201-15020B

ROBUST SUMMARY
OF INFORMATION ON

Substance Group **Kerosene/Jet Fuel**

Summary prepared by American Petroleum Institute

Creation date: May 24, 2003

Printing date: December 30, 2003

Date of last Update: **December 20, 2003**

Number of pages: **96**

NB. Reliability of data included in this summary has been assessed using the approach described by Klimisch et al.

Klimisch, H. J., Andreae, M. and Tillman, U, (1997)
A systematic approach for evaluating the quality of experimental toxicological and ecotoxicological data.
Regulatory Toxicology and Pharmacology 25, 1-5.

1.1.1 GENERAL SUBSTANCE INFORMATION

Substance type : Petroleum product
Physical status : Liquid

Remark : Kerosene is the name for the lighter end of a group of petroleum streams known as the middle distillates.
Kerosene may be obtained either from the distillation of crude oil under atmospheric pressure (straight-run kerosene) or from catalytic, thermal or steam cracking of heavier petroleum streams (cracked kerosene). The kerosenes, are further treated by a variety of processes (including hydrogenation) to remove or reduce the level of sulfur, nitrogen or olefinic materials.

The precise composition of any particular kerosene will depend on the crude oil from which it was derived and on the refinery processes used for its production. Irrespective of this, kerosenes consist predominantly of C9 to C16 hydrocarbons and boil in the range 145 to 300 °C. The major components of kerosenes are branched and straight chain paraffins and naphthenes and these normally account for 70% of the material. Aromatic hydrocarbon, mainly alkyl benzenes and alkylnaphthalenes do not normally exceed 25 % of kerosene streams. Olefins do not normally account for more than 5% of the kerosenes.

Several mammalian toxicity studies have been carried out on two samples of kerosene and the properties of these are shown below. Sample 83-09 is a straight-run kerosene and 81-07 is a hydrodesulfurized kerosene.

Property	**Sample API 83-09**	**Sample API 81-07**
CAS number	8008-20-6	64742-81-0
Pour point (°C)	-49	
Density (kg/dm³)@ 15 °C	0.81	0.82
Reid vapor pressure @ 37.8 °C (hPa)	14	
Flash point (closed cup) °C	62	60
Kinematic viscosity at 20 °C (mm²/sec)	1.5-2.5	1.1-2.5
Gravity (°API)	43.0	41.9
Sulfur (wt %)	0.47	0.07
Nitrogen (ppm)	5.4	-
Flash point (°F)	144	140
Distillation (°F)		
IBP	238	362
10%	327	392
50%	405	434
90%	475	488
95%	490	506
EP	520	535
Paraffins (%)	-	47
Saturates (%)	82	-
Olefins (%)	2.5	1
Naphthenes (%)	-	35
Aromatics (%)	15.5	18

The major use of kerosenes is as the primary ingredient in a variety of jet fuels. Kerosene is also used as diesel fuel (No. 1), domestic heating fuels (Fuel oil No. 1) and as a solvent, although this latter use is a minor one. Both diesel fuel and home heating oil No. 1 are essentially equivalent to kerosene. Specifications for both middle distillate heating fuels and transportation fuels are similar; as a consequence, it is often possible for refiners to satisfy the performance requirements of both applications with the same process stream or blend of process streams. The final products are essentially kerosene that contains additives which are specific for the intended use. Otherwise they are all virtually indistinguishable on the basis of their gross physical or chemical properties.
Characteristics of the various jet fuels are:

Jet A-1	Kerosene type used in civil aircraft, aromatic hydrocarbon content 25% (v/v) maximum, Freezing point -47°C max.
Jet A	as for Jet A-1, but with freezing point -40°C max.
AVCAT/JP-5	high flash point kerosene type used in naval aircraft, aromatic hydrocarbons 25% (v/v) max.
AVTUR/JP-8	kerosene type used in military aircraft, aromatic hydrocarbons 25 % (v/v) max.

For most of the mammalian toxicology endpoints, information has been derived on a wide range of kerosene streams and jet fuels. For simplicity, this robust summary contains detailed information on a single API sample for each endpoint and if data were available on other samples for the same endpoint they have been summarized in tabular form in the relevant sections or discussed in detail when appropriate.

1.13 REVIEWS

Memo : IARC

Remark : IARC reviewed the data on the carcinogenicity of jet fuel and concluded:

There is inadequate evidence for the carcinogenicity in humans of jet fuel.

There is inadequate evidence for the carcinogenicity in experimental animals of jet fuel.

The IARC overall evaluation was that
Jet fuel is not classifiable as to its carcinogenicity to humans.
(55)

Memo : CONCAWE

Remark : CONCAWE summarized the available health, safety and environmental data available on kerosene and jet fuels
(36)

Memo : ASTDR

Remark : A Toxicological profile for JP-5 and JP-8 jet fuel was prepared by the Agency for Toxic Substances and Disease Registry
(28)

2.1 MELTING POINT

Method : ASTM D97
GLP : No data
Test substance : Kerosene/Jet Fuels various

Remark : By definition, melting point is the temperature at which a solid becomes a liquid at normal atmospheric pressure. For complex mixtures like petroleum products, melting point may be characterized by a range of temperatures reflecting the melting points of the individual components. To better describe phase or flow characteristics of petroleum products, the pour point is routinely used. The pour point is the lowest temperature at which movement of the test specimen is observed under prescribed conditions of the test (ASTM 1999). The pour point methodology also measures a "no-flow" point, defined as the temperature of the test specimen at which a wax crystal structure and/or viscosity increase such that movement of the surface of the test specimen is impeded under the conditions of the test (ASTM 1999). Because not all petroleum products contain wax in their composition, the pour point determination encompasses change in physical state (i.e., crystal formation) and/or viscosity property. Given the pour point values given above, these products are liquid at ambient temperatures.

The pour point values for the products and streams given above are expected to represent the pour point values for the entire Kerosenes/Jet Fuel HPV category. This is expected because:

1) the products within this category consist of the same types of petroleum hydrocarbons (paraffinic, naphthenic, olefinic and aromatic),
2) the proportions of those hydrocarbon types are similar among the products/streams,
3) the hydrocarbons have a relatively similar and narrow range of molecular weights (carbon atoms of C9 - C16), and
4) the products and streams have similar distillation ranges (approximately 125 to 300 °C).

The one stream within this category which may fall outside the range of pour point values given above (i.e., CAS No. 68477-58-7) is a wider cut of petroleum hydrocarbons with a lower and higher range of molecular weight constituents (5 - 18 carbon atoms per molecule); hence, this stream would be expected to have pour points at or below those characterized by the cited data.

Result : Pour point: See following Table and Remarks Section
Decomposition: N/A
Sublimation: N/A

Product Type	Pour Point (°C)	Reference
CAS No. 8008-20-6 (Straight-run kerosene)		
	-55	API 1987
Jet A/Jet A-1 (aviation turbine fuel, kerosene type)		
	< -47	Jokuty, et al. 2002
Fuel Oil No. 1 (JP-8; kerosene)		
	-50	Jokuty, et al. 2002
Fuel Oil No. 1 (JP-5; heavy kerosene)		

< -48 Jokuty, et al. 2002

Reliability : (2) valid with restrictions
Results of standard method testing was reported in a reliable reference database.

(22) (29) (56)

2.2 BOILING POINT

Method : ASTM D86
GLP : No data
Test substance : Kerosene/Jet Fuels various

Remark : The boiling (distillation) ranges given below are expected to cover other streams and/or products in the Kerosene/Jet Fuel HPV category. This is expected due to their similar hydrocarbon content representing paraffinic, naphthenic, olefinic and aromatic constituents and their proportions within the substances in this category. Data presented below for streams not included in the Kerosene/Jet Fuel HPV category (e.g., CAS Nos. 91770-15-9 and 101316-80-7) provide supporting information to illustrate the overall similarity in boiling range for products within this category.

The one stream within the Kerosene/Jet Fuel HPV category having a larger range of molecular weight hydrocarbons than other members of the category (i.e., CAS No. 68477-58-7 with C5 to C18 carbon atoms) would be expected to have a boiling (distillation) range wider than those characterized by the cited data.

Result : Boiling point (°C): See following Table
Pressure: N/A
Pressure unit: N/A
Decomposition: N/A

	Boiling Range °C	Method	Ref.
Jet A/Jet A-1			
	145 - 300	ASTM D86	Jokuty et al. 2002
Kerosene, straight-run		API 83-09; (CAS No. 8006-20-6)	
	125 - 292	ASTM D86	API 1987
Kerosene, hydrodesulfurized		API 81-08, (CAS No. 64742-81-0)	
	175 - 284	ASTM D86	API 1987
Kerosene, sweetened (CAS No. 91770-15-9)			
	152 - 257	ASTM D86	CONCAWE 94/106
Kerosene, hydrodesulfurized (CAS No. 64742-81-0)			
	156 - 255	ASTM D86	CONCAWE 94/106
Kerosene, hydrocracked heavy aromatic (CAS No. 101316-80-7)			
	187 - 288	ASTM D86	CONCAWE 96/55

Reliability : (2) valid with restrictions
Results of standard method testing were reported in reliable review dossiers and a reference database.

(22) (39) (56)

2.4 VAPOUR PRESSURE

Method : Measured: ASTM 323
Test substance : Kerosene/Jet Fuels various

Remark : Kerosenes and jet fuels consist of complex mixtures of various hydrocarbon compounds with the majority of the structures represented by saturated hydrocarbons (e.g., normal and branched paraffins and naphthenes) with lesser amounts of aromatic compounds. Molecular weights of those constituents are represented by components containing between nine and sixteen carbon atoms (CONCAWE 1995).

Although individual hydrocarbon constituents in kerosenes exert their own vapor pressures, the vapor pressure of the mixture is the sum of the individual partial pressures of the components (Dalton's Law). The values given below are considered to be representative of the general category of kerosenes and jet fuels and the petroleum refining streams that produce them.

Result :

Vapor pressure: See table below:
Temperature: Not given
Decomposition: Not given

Vapor Pressures

kPa:	Temp (°C)	Method	Ref.
JP-5			
0.3 - 3.5	21	Not Given	Air Force (1989)
JP-8			
0.3 - 3.5	21	Not Given	Air Force (1989)
Jet A/Jet A-1			
> 1	37.8	ASTM D323	Jokuty et al.(2002)
Straight-run kerosene		CAS No. 8008-20-6	
1.4	37.8	ASTM D323	API (1987)

Reliability : (2) valid with restrictions
Results of standard method testing were reported in reliable review dossiers and a reference database.

(1) (22) (30) (36) (56)

2.5 PARTITION COEFFICIENT

Method : Calculated: KOWWIN, V1.66 (EPIWIN V3.10; EPA 2001)
Year : 2001
GLP : No
Test substance : Kerosene/Jet Fuels various

Remark : Kerosene/Jet Fuels consist principally of complex mixtures of hydrocarbon compounds having carbon chain lengths ranging from C9 to C16 (CONCAWE 1995). They consist of the following generic types of structures: straight and branched chain alkanes, naphthenes, and one to two ring aromatic hydrocarbons. Olefins may exist only in small amounts. From the carbon number range of modeled individual hydrocarbon structures, the range of Kow values of modeled representative hydrocarbon structures ranged from 3.3 to > 6 (EPA 2000).

The modeled values given below are consistent with the Kow estimates of 3.3 to >6 calculated by CONCAWE (1995) based on known hydrocarbon composition of a straight-run kerosene (CAS No. 8008-20-6) and a hydrodesulfurized kerosene (CAS No. 64742-81-0).

Result : Log Kow: 3.3 to > 6
Temperature: NA

	Log Kow Vaues	
Structure	**C9**	**C16**
n-paraffin	4.8	8.2
iso-paraffin	4.7	8.1
mono-olefin	5.2	8.1
1-ring naphthene	4.6	8.0
2-ring naphthene	3.7	7.1
3-ring naphthene	--	6.1
1-ring aromatic	3.7	7.4
2-ring aromatic	3.3(1)	6.2

(1) Value given for a C10 molecule.

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(36) (74)

2.6.1 SOLUBILITY IN DIFFERENT MEDIA

Solubility in : Water

Remark : It is predicted from solubility modeling that C9 to C16 paraffinic, naphthenic, and aromatic hydrocarbon components of kerosene/jet fuels will have approximate water solubility values of <0.001 to 52 mg/l at 25 °C. However, solubility of individual hydrocarbons decrease when comprised in the matrix of a complex petroleum mixture (Potter and Simmons 1998). For example, the solubility of pure benzene in water is approximately 1800 mg/l, whereas the concentration of benzene in water in contact with gasoline containing 1% benzene is approximately 20 mg/l (Potter and Simmons 1998). The tabulated modeled data appear consistent with and are supported by values of 20 mg/l for a sample of Jet B fuel (Jokuty et al. 2002) and 4.8 mg/l for a kerosene-type jet fuel similar to Jet A, Jet A1 and

JP-8 (Rozkov et al. 1998). Water solubility values reported here are expected to represent effective water soluble boundaries of other substances in the kerosene category based on similarities in boiling range and carbon numbers of their constituent hydrocarbon molecules.

Result : Value <0.001 mg/l to approximately 52 mg/l at 25 °C

Water Solubility Estimates, mg/l

No. C	Paraffins		Naphthenes		Aromatics	
Atoms	n-	iso-	1-ring	2-ring	1-ring	2-ring
9	2.3	2.7	3.4	19	52	31 [(1)]
16	<0.001	0.001	0.001	0.01	0.002	0.2

[(1)] Value given for C10 molecule.

Description of solubility: N/A
pH value: N/A
pKa value: N/A

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(56) (62) (63) (75)

2.14 ADDITIONAL REMARKS

Memo : Water solubility of kerosene/jet fuels

Remark : The Environmental Sciences Division of Environment Canada measured the water solubility for a wide-cut aviation fuel (Jet B) to be 20.34 mg/l (Jokuty et al. 2002). Solubility measurements were made by placing 10 ml of fuel on top of 100 ml of fresh water in a 125-ml separatory funnel. This was allowed to stand for one week, in the dark, at room temperature. The analysis was done by purge and trap using a Hewlett-Packard 5890 gas chromatograph and a flame ionization detector (FID) with a Tekmar 4000 Dynamic Headspace Concentrator. A trap containing 3% SP-2100/Chromosorb W Aw and Tenax TA (Supelco) was used, together with the following parameters:

Tekmar 4000

Purge pressure	20 psi
Purge gas (helium) flow	40 ml/minute
Purge time	12 minutes
Desorb time	4 minutes
Line temperature	115 - 120 °C
Valve temperature	115 - 120 °C

Gas Chromatograph

Initial temperature	40 °C
Initial time	10 minutes
Heating rate	6 °C/minute
Final temperature	250 °C
Detector temperature	300 °C

Source : Jokuty, P., S. Whiticar, Z. Wang, M. Fingas, B. Fieldhouse, P. Lambert, and J. Mullin (2002)
Properties of Crude Oils and Oil Products.
Environmental Protection Service, Environment Canada, Ottawa, Ontario.
Internet Version 2002 via http://www.etcentre.org/spills.

Reliability : (2) valid with restrictions

Memo : Water solubility of kerosene/jet fuels

Remark : Groundwater contaminated with aviation fuel similar to commercial civil kerosenes Jet A, Jet A-1, and JP-8 was measured for dissolved hydrocarbon components. Water soluble fractions of non-polar hydrocarbons were 4.78 mg/l. Concentrations of hydrocarbons were extracted from water with 10 ml of pentane per 1 liter of sample for 1 hour then measured by GC/MS. The gas chromatograph was a Varian 3400 CX equipped with an OV-101 column (30 m length, 0.25 mm id) packed with DB-1 (1.0 µm dia particle). A flame ionization detector (FID) was used. Operating conditions were:

Flow rate (nitrogen)	3 ml/min
Air	350 ml/min
Hydrogen	30 ml/min
Make-up gas (nitrogen)	25 ml/min

Detector Conditions:

Oven temperature (1st phase)	40 °C for 2 min; increase 25 °C/min over 7.2 min 220 °C hold for 1 min.
Oven temperature (2nd phase)	15 °C/min to 300 °C

Detection limits:

Single components	0.1 µg/l
Total hydrocarbons	3 µg/l
Precision	0.05 µg/l

Source : Rozkov, A., A. Kaard, and R. Vilu (1998)
Biodegradation of dissolved jet fuel in chemostat by a mixed bacteria culture isolated from a heavily polluted site.
Biodegradation 8:363.-369.

Reliability : (2) valid with restrictions

Memo : Water solubility of kerosene/jet fuels

Remark : ATSDR (1998) cited a water solubility value of approximately 5 mg/l at 20 °C for JP-5/JP-8 (kerosene) that had been reported by the Air Force (1989).

Source : Air Force (1989)
The installation restoration program toxicology guide. Oak Ridge, TN: Biomedical and Environmental Information Analysis. Vol. 4. [as cited in ATSDR (Agency for Toxic Substances and Disease Registry). 1998. Toxicological Profile for JP-5 and JP-8. U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia].

Reliability : (4) not assignable

Memo : Water solubility of kerosene/jet fuels

Remark : ATSDR (1995) cited a water solubility value of approximately 57 mg/l at 20 °C for a "wide-cut" aviation fuel, JP-4, that had been reported by CRC (1984).

Source : CRC (1984)
Handbook of aviation fuel properties. Atlanta, GA: Coordinating Research Council, Inc. Report no. 53.

[as cited in ATSDR (Agency for Toxic Substances and Disease Registry). 1995. Jet fuels (JP-4 and JP-7). Toxicological Profile. ATSDR Toxicological Profiles, U.S. Department of Health and Human Services, Public Health Service, Atlanta, Georgia].

Reliability : (4) not assignable

3.1.1 PHOTODEGRADATION

Method : Calculated: by AOPWIN V1.90. (EPIWIN V3.10; EPA 2000)
Year : 2000
GLP : No
Test substance : Kerosene/Jet Fuels various

Remark : Direct photolysis is not expected to be a major degradation pathway for many of the components in kerosene/jet fuels. Chemicals having the potential to photolyze have UV/visible absorption maxima in the range of 290 to 800 nm. Most hydrocarbon constituents in this category are not expected to photolyze since they do not show absorbance within the 290-800 range. These include paraffinic, naphthenic and one-ring alkylbenzene compounds. However, degradation of polyaromatic hydrocarbons by reaction with sunlight in the presence of oxygen can be a significant removal process where such substances are present in, or near the surface of water (CONCAWE 2001). These include components in kerosene/jet fuels having two or more aromatic ring structures.
It is predicted from indirect photolysis modeling of C9 to C16 paraffinic, naphthenic, olefinic and aromatic hydrocarbon compounds that volatile components in gas oils will undergo moderate to rapid atmospheric oxidation and not persist in the environment.

Result : Concentration of substance: N/A
Temperature: 25 °C
Direct photolysis: Many substances in this category are not subject to direct photolysis; see remarks section, below.
Half life: N/A
Degradation %: N/A
Quantum yield: N/A
Indirect Photolysis:
Sensitizer type: Hydroxyl radicals (OH^-)
Concentration of sensitizer: 1.5×10^6 OH^-/cm^3
Rate constant: various
Half-life T ½: see table of half-lives below (values given in days):

	HALF LIVES, days	
	C9	**C16**
n-paraffins	1.1	0.5
Iso-paraffins	1.1	0.5
1-ring napthenes	0.8	0.5
2-ring naphthenes	0.8	0.4
3-ring naphthenes	0.4 (1)	0.3
1-ring aromatics	1.5	0.6
2-ring aromatics	0.5 (2)	0.2
3-ring aromatics	0.3 (1)	0.3

(1) Value given for 14 carbon structure
(2) Value given for 10 carbon structure

Breakdown products: N/A

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model.
(41) (73)

3.1.2 STABILITY IN WATER

Test substance : Kerosene/Jet Fuels various

Remark : Hydrolysis of an organic chemical is the transformation process in which a water molecule or hydroxide ion reacts to form a new carbon-oxygen bond. Chemicals that have a potential to hydrolyze include alkyl halides, amides, carbamates, carboxylic acid esters and lactones, epoxides, phosphate esters, and sulfonic acid esters. The chemical components that comprise the gas oil category are hydrocarbons that are not subject to hydrolysis because they lack functional groups that hydrolyze.

Result :

Nominal:	N/A
Measured:	N/A
Half-life T1/2:	N/A
Degradation %:	N/A
At specified pH:	N/A
Breakdown Products:	N/A

Reliability : (1) valid without restriction

(54)

3.3.1 TRANSPORT BETWEEN ENVIRONMENTAL COMPARTMENTS

Method : Calculated by fugacity-based Environmental Equilibrium Partitioning Model (EQC model)

Remark : Multimedia distribution was calculated for low and high molecular weight hydrocarbon compounds representing common PONA (i.e., paraffinic, naphthenic and aromatic) constituents in Kerosene/Jet Fuels. Partitioning behavior depends largely on molecular weight, with smaller compounds (e.g., 9 carbon atoms) partitioning to the air due to relatively high vapor pressures. Here they are expected to degradation rapidly via hydroxyl radical attack (indirect photodegradation). Larger molecular weight hydrocarbons (e.g., 16 carbon atoms) tend to partition to the terrestrial environment or to air where they are expected to undergo moderate to slow biodegradation and indirect photodegradation, respectively. Distribution in the aquatic environment is low due to low water solubility or to high vapor pressure.

Result :

	PERCENT DISTRIBUTION					
	Air	**Water**	**Soil**	**Sed.**	**Susp. Sed.**	**Fish**
n-paraffins						
C9	99	<0.1	1	<0.1	<0.1	<0.1
C16	6	<0.1	92	2	<0.1	<0.1
Iso-paraffins						
C9	99	<0.1	0.5	<0.1	<0.1	<0.1
C16	60	<0.1	39	0.9	<0.1	<0.1
1-ring naphthenes						
C9	99	<0.1	0.9	<0.1	<0.1	<0.1
C16	5	<0.1	93	2	<0.1	<0.1
2-ring naphthenes						
C9	99	0.2	1	<0.1	<0.1	<0.1
C16	34	<0.1	65	1	<0.1	<0.1
3-ring naphthenes						
C14	71	0.2	28	0.6	<0.1	<0.1

C16	40	<0.1	59	1	<0.1	<0.1
1-ring aromatics						
C9	97	1	2	<0.1	<0.1	<0.1
C16	13	<0.1	85	2	<0.1	<0.1
2-ring aromatics						
C10	77	8	15	0.3	<0.1	<0.1
C16	1	<0.1	97	2	<0.1	<0.1
3-ring aromatics						
C14	1	4	93	2	0.1	<0.1
C16	0.7	0.5	97	2	<0.1	<0.1

Reliability : (2) valid with restrictions
The predicted endpoint was determined using a validated computer model. (57)

3.5 BIODEGRADATION

Type : Aerobic
Inoculum : Activated sludge
Contact time : 28 day(s)
Method : OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year : 1999
GLP : No
Test substance : Nigerian Diesel Fuel; CAS No. 68334-30-5

Remark : This diesel fuel stream did not satisfy the test criteria for ready degradability of 60% degradability within 28 days. Although this diesel fuel is not considered 'readily' biodegradable, it is inherently biodegradable since significant degradation did occur, based on EPA guidance for using ready and inherent biodegradability tests (http://www.epa.gov/oppt/exposure/docs/halflife.htm.)

Result : Average biodegradation for duplicate test flasks at 28 days:
diesel fuel = 57.5%
rapeseed oil = 84.4% (satisfied positive control criteria).
The report noted that the oxygen consumption of the blank controls was below 60 mg/l as required by the test guidelines.

Test condition : Activated sludge inoculum for the test was collected from Medford Municipal Wastewater Treatment Plant in Medford, NJ. The plant treats predominately domestic sewage. To reduce background oxygen consumption, the activated sludge was aerated for approximately 27 hours prior to use. Sufficient supernatant was decanted to provide a 1% (v/v) inoculum for each respirometry vessel. The sewage inoculum had a microbial density of $1E^4$ colony forming units per ml, as measured using a commercial dip-slide method, and was within the guideline criteria of $1E^7$-$1E^8$ CFU/l.
Test flasks contained 990 ml of inoculated mineral salts medium , ca. 25 mg of test substance and 10 ml of sludge supernatant (inoculum). Also included in the experiment were duplicate blank flasks containing inoculated medium and duplicate reference substance flasks containing inoculated medium and 25 mg of low erucic acid rapeseed oil (LEAR).
The test was run at 22 °C (± 1°C) using a C.E.S. Aerobic Respirometer for 28 days. The instrument determined the oxygen demand every hour and replenished the oxygen through the electrolysis of copper sulfate.

The extent of biodegradation over 28 days was calculated as the measured biochemical oxygen demand (BOD) expressed as a percentage of the ThOD (theoretical oxygen demand).

Reliability : (1) valid without restriction

(58)

Type : Aerobic
Method : ASTM D2329-68
Year : 1971
GLP : No
Test substance : Kerosene, hydrotreated Avtur (Aviation turbine fuel)

Method : BOD test method using a closed-system respirometer apparatus. Referenced as ASTM D2329-68

Inoculum was freshwater used activated aerobic sludge obtained from a laboratory scale activated sludge plant. Seawater: naturally-occurring bacteria in North Sea saltwater

Remark : Note: Test design details were minimal -no information on test systems (i.e., replicates, blank control O_2 data) were included in the citation, no water chemistry reported, no discussion on TOD determination for substrates included. However glucose (positive control) 5 day results were within required criteria for both fresh (70%) and saltwater media (55%) containing nutrient solution. Also oleic acid, a sparingly soluble oil, but easily degradable substrate achieved comparable degradation compared to glucose, with 67% (freshwater) and 53% degradation (seawater) after 5 day incubation at 30 °C. Additionally, when testing these two rapidly degradable substrates in seawater with no nitrogen or phosphorus amendments, degradation was significantly reduced at 5 days, with only 30% of the glucose and 13% of oleic acid degraded. Based on the performance for these rapidly degradable substances, the reliability of the experimental results are acceptable.

Result : After 10 days, kerosene was degraded up to 46% in freshwater, while kerosene was degraded in seawater up to 53% and 2%, respectively, with and without nitrogen and phosphorus amendments.

The results of biodegradation testing of kerosene in freshwater and seawater are as follows:

Substrate/Medium	Nutrients Added	BOD/TOD, % 5 days	10 days
Kerosene			
Fresh water	+	41	46
Sea water	+	36	53
Sea water	-	2	2

Test condition : Fresh water amended with nutrient salts as per ASTM method plus 3 ml of effluent from a laboratory scale activated sludge plant (adapted to consumption of oil) was spiked with approximately 20 - 30 mg/l kerosene. Cell counts of sludge effluent were not reported. Seawater was sampled from the North Sea about 10 miles west of Den Helder, Netherlands. Average seawater salinity was 31.8 g/l and an average of 200-500 cells/ml bacteria was determined with the membrane filter technique. Seawater experiments were run with and without amendments of nitrogen (as ammonia) and phosphorous (as phosphate) in varying concentrations of each. Fresh water experiments were run with N and P amendments according the official method. Tests in both freshwater and seawater were conducted at 30 °C.

Conclusion	:	Experimental results indicate that the rate of degradation was highly dependent on the available nitrogen and phosphorus for a variety of substrates, but was critical in influencing the extent of kerosene degradation in sea water.
Reliability	:	(2) valid with restrictions Acceptable, well-documented publication which meets basic scientific principles.

(33)

Inoculum	:	Activated sludge
Contact time	:	28 day(s)
Method	:	OECD Guide-line 301 F "Ready Biodegradability: Manometric Respirometry Test"
Year	:	1999
GLP	:	No
Test substance	:	Kerosene Mid-Blend; CAS No. 8008-20-6
Remark	:	This kerosene stream did not satisfy the test criteria for ready degradability of 60% degradability within 28 days. Although this kerosene stream is not considered 'readily' biodegradable, it is inherently biodegradable since significant degradation did occur, based on EPA guidance for using ready and inherent biodegradability tests (http://www.epa.gov/oppt/exposure/docs/halflife.htm.)
Result	:	Average biodegradation for duplicate test flasks at 28 days: kerosene = 58.6% rapeseed oil = 84.4% (satisfied positive control criteria). The report noted that the oxygen consumption of the blank controls was below 60 mg/l as required by the test guidelines.
Test condition	:	Activated sludge inoculum for the test was collected from Medford Municipal Wastewater Treatment Plant in Medford, NJ. The plant treats predominately domestic sewage. To reduce background oxygen consumption, the activated sludge was aerated for approximately 27 hours prior to use. Sufficient supernatant was decanted to provide a 1% (v/v) inoculum for each respirometry vessel. The sewage inoculum had a microbial density of $1E^{+4}$ colony forming units per ml, as measured using a commercial dip-slide method, and was within the guideline criteria of $1E^{7}$-$1E^{8}$ CFU/l. Test flasks contained 990 ml of inoculated mineral salts medium , ca. 25 mg of test substance and 10 ml of sludge supernatant (inoculum). Also included in the experiment were duplicate blank flasks containing inoculated medium and duplicate reference substance flasks containing inoculated medium and 25 mg of low erucic acid rapeseed oil (LEAR). The test was run at 22 °C (± °C) using a C.E.S. Aerobic Respirometer for 28 days. The instrument determined the oxygen demand every hour and replenished the oxygen through the electrolysis of copper sulfate. The extent of biodegradation over 28 days was calculated as the measured biochemical oxygen demand (BOD) expressed as a percentage of the ThOD (theoretical oxygen demand).
Reliability	:	(1) valid without restriction

(59)

3.8 ADDITIONAL REMARKS

Memo : Biodegradability of kerosene/jet fuels

Remark : Removal of dissolved hydrocarbon components in water soluble fractions of aviation jet fuel was >99% for two dilution rates in a chemostat culture.

Biodegradation of water soluble fractions of kerosene-type aviation fuel (e.g., Jet A, Jet A1, and JP-8) were measured in a chemostat culture apparatus. A mixed microbial culture was isolated from groundwater contaminated with aviation fuel. Inoculum for the experiments was pre-grown for three days in culture medium of basal medium M9 diluted 10-fold by distilled water plus trace elements and fuel (1% v/v). Feeding medium for the chemostat was prepared using basal medium M9 diluted 10-fold by distilled water and autoclaved. Jet fuel was added after autoclaving in the ratio 5:1000 (0.5%). The mixture was stirred for 5 hours, and the insoluble part was separated and discarded. Cultivations were carried out in 2-l fermentators. The ratio of inoculation was 1:10, with the volume in the fermentator maintained at 1 l by an overflow device. Experiments were run for 113 hours using fermentator volumes of 11.3 and 19.4 for dilution rates of 0.1/h and 0.17/h, respectively. An abiotic control was run under the same environmental conditions except that the solution held sodium azide (2000 mg/l) to prevent bacterial growth. Experiments were run at 27 °C. The concentrations of soluble hydrocarbons before and after treatment in the chemostat were measured by GC/MS.

Residual nonpolar hydrocarbon concentrations were 2.1 µg/l and 0.42 µg/l in experiments with dilution rates of 0.17/h and 0.1/h, respectively. The measured nonpolar hydrocarbon concentration in the chemostat culture medium was 4.78 mg/l. Removal achieved >99% for both dilution rates.

Source : Rozkov, A., A. Kaard, and R. Vilu (1998) Biodegradation of dissolved jet fuel in chemostat by a mixed bacterial culture isolated from a heavily polluted site. Biodegradation. 8:363-369.

Reliability : (2) valid with restrictions

4.1 ACUTE/PROLONGED TOXICITY TO FISH

Type	:	Semistatic
Species	:	Oncorhynchus mykiss (Fish, fresh water)
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year	:	1995
GLP	:	Yes
Test substance	:	CAS No. 91770-14-9; Kerosene (petroleum), sweetened

Method : LL_{50} values calculated using binomial and Trimmed Spearman-Karber methods; the NOEL was determined using Dunnett's Test.

Remark : The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, the measurements at the nominal level of 4.5 mg/l and below were extrapolations outside the calibration range.

Result : At the end of the test, all fish of the control, and the 0.36, 0.9, and 4.5 mg/l WAF solutions were alive and showed no abnormal effects. Fish exposed to the 23 mg/l WAF solution had 53% mortality with abnormal effects of dark pigmentation and/or lethargic in the surviving fish. All fish exposed to the 50 mg/l WAF solution died during the first 24 hours of the test.

The LL_{50} values with confidence intervals were:

Exposure Time, hours	LL_{50}, mg/l	Confidence Interval, mg/l
24	36	23 - 50
48	23	17 - 30
72	19	14 - 26
96	18	13 - 24

The maximum nominal loading rate causing no mortality was 4.5 mg/l.
The minimum nominal loading rate causing 100% mortality was 50 mg/l.
The No-Observed-Effect Level (NOEL) was 4.5 mg/l.

Chemical analyses of the test solutions showed (mg/l as naphthalene):

	Treatment level					
	Control	**0.36**	**0.9**	**4.5**	**23**	**50**
Day 0						
new	.0099	.0078	.015	.30	1.6	--
Day 1						
old	.0084	.0073	.015	.027	1.5	3.4
new	.017	.011	.022	.43	1.9	--
Day 2						
old	ND	ND	.033	.33	1.7	--
new	ND	ND	.22	.41	1.8	--
Day 3						
old	ND	ND	.12	.36	1.6	--
new	ND	.039	.05	.41	1.9	--
Day 4						
old	ND	ND	.017	.3	1.2	--

ND = not detected
-- means all fish dead, no further measurements were made.

Deviations were noted for:
1) to maintain dissolved oxygen in close vessel, slightly smaller fish than guideline recommendations were used,
2) temperature 0.3 °C above limit for a brief time during the holding period,
3) temperature 0.4 °C above maximum limit for 5 hours during testing
4), due to a calculation error, one of the test levels was 0.36 mg/l rather than the intended 0.2 mg/l,
5) test substance was not held under refrigerated storage for approximately 3 weeks.

Test condition : Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs, an appropriate volume of test substance was added quantitatively via syringe to glass carboys holding dilution water. The glass carboys were stoppered then the mixtures stirred for 24 hours (10% vortex, approximately 1-2 cm). An equilibration study run prior to the test justified a 24-hour stirring period. After a 1-hour settling period, WAF solutions were siphoned from the bottom of the carboy into glass test chambers. Test chambers were 4-liter (nominal) glass aspirator bottles. Bottles were completely filled with no headspace when used in the test. Three replicate test bottles were prepared for each treatment level. Nominal loading rates used in the test were 0 (control), 0.36, 0.9, 4.5, 23, and 50 mg/l. No undissolved test substance was observed in any of the WAF solutions during the test.

Test fish were juvenile rainbow trout (approximately 5 weeks old) originating from a commercial supplier (Thomas Fish Company, Anderson, CA) and fed a commercial fish diet (Ziegler Bros., Inc., Gardners, PA and Tetramin®) during the holding period. They were held in dilution water at least 12 days and at approximately 15 °C for at least 7 days prior to use in testing. Mortality during the holding period was insignificant. Food was withheld approximately 24 hours before testing. Fish used in the test measured 2.8 cm (sd = 0.3) mean total length and 0.162 g (sd = 0.05) mean weight.

To start the test, individual fish were randomly selected one at a time and placed into intermediate holding chambers, one for each test chamber, until each holding chamber contained five fish. Fish were then transferred to their respective test chambers, which then were sealed with no headspace. Biomass loading in the test chambers was 0.18 g/l.

Samples of the WAF solutions were collected on Days 0, 1, 2, 3, and 4 for chemical analysis. Freshly-prepared WAFs were collected from the mixing vessels while 24-hour old WAFs were collected as a composite from all replicates of a treatment level. WAF samples were extracted with hexane and analyzed by Gas Chromatography using a Flame Ionization Detector (GC-FID) and a capillary column. Measured concentrations were presented in the report as mg/l as naphthalene.

The test was conducted in a temperature-controlled area under a 16 hour/8 hour, light/dark photoperiod. Daylight intensity ranged from 70 to 71 foot-candles. Measurements of fresh solutions for water quality parameters showed dissolved oxygen concentration ranged from 8.3 to 9.9 mg/l, pH ranged from 7.9 to 8.7, and temperature ranged from 14 to 15 °C. Measurements of old solutions showed dissolved oxygen concentration

ranged from 6.8 to 8.5 mg/l and pH ranged from 7.7 to 8.3. The temperature of the old solutions was not measured.

Dilution water was a mixture of carbon filtered well water and water dialyzed by reverse osmosis. This water had an alkalinity of 63 - 65 mg $CaCO_3$/l, hardness of 180 - 220 mg $CaCO_3$/l, specific conductance of 380 - 410 µmhos, pH of 7.5 - 7.7, and a dissolved oxygen concentration of 8.7 - 10 mg/l at the beginning and end of the test. Total organic carbon measured 1.3 ppm during the monthly screening.

Reliability : (1) valid without restriction

(48)

Type : Semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1995
GLP : Yes
Test substance : CAS No. 64742-81-0; kerosene (petroleum), hydrodesulfurized

Method : LL_{50} values calculated using the graphical and binomial methods; the NOEL was determined using Dunnett's Test.

Remark : The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, the measurements at the nominal level of 4.5 mg/l and below were extrapolations outside the calibration range.

Result : At the end of the test, all fish of the control, and the 0.3, and 1.4 mg/l WAF solutions were alive and showed no abnormal effects. Fish exposed to the 6.8 mg/l WAF solution had 0% mortality with abnormal effects of dark pigmentation or erratic swimming in 27% of the surviving fish. Seventy-three percent of the fish showed no abnormal effects. Fish exposed to the 34 mg/l WAF solution had 100% mortality by 72 hours. Fish exposed to the 75 mg/l WAF solution had 93% mortality by 96 hours, with one surviving fish showing abnormal effects of dark pigmentation and lethargic.

The LL_{50} values with confidence intervals were:

Exposure Time, hours	LL_{50}, mg/l	Confidence Interval, mg/l
24	16	could not calculate
48	16	could not calculate
72	20	6.8 - 34
96	20	6.8 - 34

The maximum nominal loading rate causing no mortality was 6.8 mg/l.
The minimum nominal loading rate causing 100% mortality was 34 mg/l.
The No-Observed-Effect Level (NOEL) was 6.8 mg/l.

Chemical analyses of the test solutions showed (mg/l as naphthalene):

	Treatment level					
	Control	**0.3**	**1.4**	**6.8**	**34**	**75**
Day 0						
new	.0091	.047	.13	.12	4.6	3
Day 1						
old	.099	.034	.17	.66	4	2.6
new	.0091	.017	.12	.83	3.1	2.5
Day 2						
old	.014	.0023	.063	.68	2.8	2.5
new	.0021	.0029	.11	.77	1.8	5.4
Day 3						
old	.072	.072	.12	.83	1.2	5.7
new	.014	.039	.15	.53	2.8	4.6
Day 4						
old	.0025	.002	.038	.59	--	4.5

ND = not detected
-- = All fish dead at this concentration, no measurements taken.

Deviations were noted for:

1) to maintain dissolved oxygen in close vessel, slightly smaller fish than guideline recommendations were used
2) temperature excursions of 0.1 and 0.2 °C above limit for two brief times during the holding period
3) dissolved oxygen concentrations fell below 60% saturation on several occasion during the definitive test. The deviation ranged from 0.5 to 1.1 mg/l below the minimum limit (5.9 mg/l at 16 °C)
4) test substance was not held under refrigerated storage for approximately 3 weeks
5) biological observations were made 7 minutes outside the timeframe of ±1 hour
6) the loading rates assigned for the definitive test were not in a contiguous geometric series.

Test condition : Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs, an appropriate volume of test substance was added quantitatively via syringe to glass carboys holding dilution water. The glass carboys were stoppered then the mixtures stirred for 24 hours (10% vortex, approximately 1-2 cm). An equilibration study run prior to the test justified a 24-hour stirring period. After a 1-hour settling period, WAF solutions were siphoned from the bottom of the carboys into glass test chambers. Test chambers were 4-liter (nominal) glass aspirator bottles. Bottles were completely filled with no headspace when used in the test. Three replicate test bottles were prepared for each treatment level. Nominal loading rates used in the test were 0 (control), 0.3, 1.4, 6.8, 34, and 75 mg/l. No undissolved test substance was observed in any of the WAF solutions during the test.

Test fish were juvenile rainbow trout (approximately 6.5 weeks old) originating from a commercial supplier (Thomas Fish Company, Anderson, CA) and fed a commercial fish diet (Ziegler Bros., Inc., Gardners, PA and Tetramin®) during the holding period. They were held in dilution water at least 12 days and at approximately 15 °C for at least 7 days prior to use in

testing. Mortality during the holding period was insignificant. Food was withheld approximately 24 hours before testing. Fish used in the test measured 3.6 cm (sd = 0.2) mean total length and 0.377 g (sd = 0.057) mean weight.

To start the test, individual fish were randomly selected one at a time and placed into intermediate holding chambers, one for each test chamber, until each holding chamber contained five fish. Fish were then transferred to their respective test chambers, which then were sealed with no headspace. Biomass loading in the test chambers was 0.42 g/l.

Samples of the WAF solutions were collected on Days 0, 1, 2, 3, and 4 for chemical analysis. Freshly-prepared WAFs were collected from the mixing vessels while 24-hour old WAFs were collected as a composite from all replicates of a treatment level. WAF samples were extracted with hexane and analyzed by Gas Chromatography using a Flame Ionization Detector (GC-FID) and a capillary column. Measured concentrations were presented in the report as mg/l as naphthalene.

The test was conducted in a temperature-controlled area under a 16 hour/8 hour, light/dark photoperiod. Daylight intensity ranged from 67 to 68 foot-candles. Measurements of fresh solutions for water quality parameters showed dissolved oxygen concentration ranged from 8.0 to 9.8 mg/l, pH ranged from 7.5 to 8.7, and temperature ranged from 15.6 to 16.0 °C. Measurements of old solutions showed dissolved oxygen concentration ranged from 4.8 to 8.0 mg/l and pH ranged from 7.1 to 8.1. The temperature of the old solutions was not measured.

Dilution water was a mixture of carbon filtered well water and water dialyzed by reverse osmosis. This water had an alkalinity of 64 - 70 mg $CaCO_3$/l, hardness of 170 - 174 mg $CaCO_3$/l, specific conductance of 380 - 420 µmhos, pH of 7.6, and a dissolved oxygen concentration of 8.5 - 8.8 mg/l at the beginning and end of the test. Total organic carbon measured 1.3 ppm during the monthly screening.

Reliability : (1) valid without restriction

(52)

Type : Semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1994
GLP : Yes
Test substance : CAS No. 64742-81-0, Kerosene, hydrotreated

Method : Visual inspection
Result : 96-hr LL_{50} lies between 10-100 mg/l WAF, based on nominal loading rates. Mortality at 96 hrs was 0, 0, 100, and 100% in the 0, 10, 100, and 1000 mg/l treatments.
Only four concentrations were tested which is less than a minimum of five concentrations stated in the guidelines. Water hardness was higher than targeted range of 50 - 250 mg/l as $CaCO_3$. Hardness range of 284 - 288 mg/l as $CaCO_3$ is normal for this laboratory and does not affect the survival of the fish.

Analytical method used was gas chromatography-mass spectrometry. Mean reduction in the concentration of dissolved components of kerosene for each 24-hr period was 40%, 19%, and 7% for the 10, 100, and 1000 mg/l WAFs.

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 10, 100, and 1000 mg/l. Control and dilution water was laboratory mains tap water obtained from bore holes, and passed through particle and activated carbon filters (alkalinity 252 mg/l as $CaCO_3$, hardness 277 mg/l as $CaCO_3$, conductivity 520 S/cm, pH 7.4). Test substance was mixed in dilution water for ~70 hrs in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MS. Mixtures were allowed to settle 1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 12-liter glass aspirators completely filled with test solution and contained 7 fish per vessel. Test fish had a mean length of 4.5 cm and a mean weight of 0.83 g. Fingerlings were obtained from Zeals Trout Farm, Zeals, Wiltshire, U.K, and acclimated to test conditions for more than 9 days before use. One replicate per treatment and control were used. Test solutions were renewed daily with surviving fish transferred to the freshly prepared WAFs. Test temperature was 14 - 15 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was >60% saturation (8.5 to 9.8 mg/l). pH was 7.3 - 7.8.
To monitor the concentration of dissolved components in the test solutions, samples were collected at the beginning and end of each 24-hr period for each of the batches of WAFs prepared during the 96-hr test.

Reliability : (1) valid without restriction

(70)

Type : Semistatic
Species : Oncorhynchus mykiss (Fish, fresh water)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : OECD Guide-line 203 "Fish, Acute Toxicity Test"
Year : 1995
GLP : Yes
Test substance : CAS No. 101316-80-7; Solvent naphtha (petroleum), hydrocracked heavy aromatic

Method : Statistical method:
LL_{50} values calculated using the probit method; the NOEL was determined using Dunnett's Test.

Remark : The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, most of the measurements at the nominal level of 6.8 mg/l and below were extrapolations outside the calibration range.

Result : At the end of the test, all fish of the control, and the 0.3, and 1.4 mg/l WAF solutions were alive and showed no abnormal effects. Fish exposed to the 6.8 mg/l WAF solution had 6.7% mortality with abnormal effects of dark pigmentation and/or lethargic in 50% of the surviving fish. Fifty percent of the surviving fish showed no abnormal effects. Fish exposed to the 34 mg/l WAF solution had 80% mortality with abnormal effect of lethargic in all surviving fish. Fish exposed to the 75 mg/l WAF solution had 95% mortality by 48 hours, and all fish were dead by the end of the test.

The LL_{50} values with confidence intervals were:

Exposure Time, hours	LL_{50} mg/l	Confidence Interval, mg/l
24	could not calculate	
48	42	33 - 53
72	38	30 - 49
96	25	20 - 32

The maximum nominal loading rate causing no mortality was 1.4 mg/l.
The minimum nominal loading rate causing 100% mortality was 75 mg/l.
The No-Observed-Effect Level (NOEL) was 6.8 mg/l.

Chemical analyses of the test solutions showed (mg/l as naphthalene):

	Treatment level					
	Control	0.3	1.4	6.8	34	75
Day 0						
new	.029	.0026	.024	.5	1.9	2.8
Day 1						
old	.0014	.00073	.014	.35	1.7	2.7
new	ND	.00074	.083	.32	2.1	3.2
Day 2						
old	.0058	.069	.032	.29	1.7	2.9
new	.029	.041	.035	.91	2.4	3.2
Day 3						
old	.021	.012	.047	.61	1.4	2.9
new	.0014	.0021	.031	.47	1.9	3.2
Day 4						
old	.031	.0091	.041	.41	1.8	3.3

ND = not detected

Deviations were noted for:

1) on day 2, a 0.1 mg/l solution was used to renew the 0.3 mg/l treatment. This was observed and corrected within 30-45 minutes
2) to maintain dissolved oxygen in close vessel, slightly smaller fish than guideline recommendations were used
3) temperature 0.1 °C above limit for a brief time during the holding period
4) Dissolved oxygen concentrations fell below 60% saturation on several occasions during the definitive test. The deviation ranged from 0.1 to 0.7 mg/l below the minimum limit (5.9 mg/l at 16 °C)
5) test substance was not held under refrigerated storage for approximately 3 weeks
6) the loading rates assigned for the definitive test were not in a contiguous geometric series.

Test condition : Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs, an appropriate volume of test substance was added quantitatively via syringe to glass carboys holding dilution water. The glass carboys were stoppered then the mixtures stirred for 24 hours (10% vortex, approximately 1-2 cm). An equilibration study run prior to the test justified a 24-hour stirring period. After a 1-hour settling period, WAF solutions were siphoned from the bottom of the carboys into glass test chambers. Test chambers were 4-liter (nominal) glass aspirator bottles. Bottles were completely filled with no

headspace when used in the test. Three replicate test bottles were prepared for each treatment level. Nominal loading rates used in the test were 0 (control), 0.3, 1.4, 6.8, 34, and 75 mg/l. No undissolved test substance was observed in any of the WAF solutions during the test.

Test fish were juvenile rainbow trout (approximately 6.5 weeks old) originating from a commercial supplier (Thomas Fish Company, Anderson, CA) and fed a commercial fish diet (Ziegler Bros., Inc., Gardners, PA and Tetramin®) during the holding period. They were held in dilution water at least 12 days and at approximately 15 C for at least 7 days prior to use in testing. Mortality during the holding period was insignificant. Food was withheld approximately 24 hours before testing. Fish used in the test measured 3.9 cm (sd = 0.4) mean total length and 0.432 g (sd = 0.113) mean weight.

To start the test, individual fish were randomly selected one at a time and placed into intermediate holding chambers, one for each test chamber, until each holding chamber contained five fish. Fish were then transferred to their respective test chambers, which then were sealed with no headspace. Biomass loading in the test chambers was 0.48 g/l.

Samples of the WAF solutions were collected on Days 0, 1, 2, 3, and 4 for chemical analysis. Freshly-prepared WAFs were collected from the mixing vessels while 24-hour old WAFs were collected as a composite from all replicates of a treatment level. WAF samples were extracted with hexane and analyzed by Gas Chromatography using a Flame Ionization Detector (GC-FID) and a capillary column. Measured concentrations were presented in the report as mg/l as naphthalene.

The test was conducted in a temperature-controlled area under a 16 hour/8 hour, light/dark photoperiod. Daylight intensity ranged from 55 to 68 foot-candles. Measurements of fresh solutions for water quality parameters showed dissolved oxygen concentration ranged from 7.8 to 9.6 mg/l, pH ranged from 7.8 to 8.7, and temperature measured a constant 16 °C. Measurements of old solutions showed dissolved oxygen concentration ranged from 5.2 to 7.8 mg/l and pH ranged from 7.1 to 8.2. The temperature of the old solutions was not measured.

Dilution water was a mixture of carbon filtered well water and water dialyzed by reverse osmosis. This water had an alkalinity of 64 - 70 mg $CaCO_3$/l, hardness of 170 - 174 mg $CaCO_3$/l, specific conductance of 380 - 420 µmhos, pH of 7.6, and a dissolved oxygen concentration of 8.5 - 8.8 mg/l at the beginning and end of the test. Total organic carbon measured 1.3 ppm during the monthly screening.

Reliability : (1) valid without restriction

(50)

4.2 ACUTE TOXICITY TO AQUATIC INVERTEBRATES

Type	:	Static renewal
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit Test	:	no
Analytical monitoring	:	yes
Method	:	OECD Guide-line 202

Year : 1995
GLP : yes
Test substance : CAS No. 91770-14-9; Kerosene (petroleum), sweetened

Method : EL_{50} values calculated using graphical and probit methods, the NOEL was determined by Dunnett's Test.
Remark : The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, the measurements at the nominal level of 4.5 mg/l and below were extrapolations outside the calibration range.
Result : At the end of the test, one individual from the control group was immobile, all other daphnids of the control group appeared normal. Daphnids exposed to the 0.1, 0.36, and 0.9 mg/l WAF solutions were alive and appeared normal. Daphnids exposed to the 4.5 mg/l WAF solution had 15% immobilization, with surviving daphnids appearing normal. Daphnids exposed to the 23 mg/l WAF solution had 55% immobilization with surviving daphnids appearing normal. All daphnids exposed to the 50 mg/l WAF solution were immobile at the end of the test.

The EL_{50} values with confidence intervals were:

Exposure Time, hours	EL_{50}, mg/l	Confidence Interval, mg/l
24	46	could not be calculated
48	21	17 - 27

The maximum nominal loading causing no immobilization was 0.9 mg/l. The minimum nominal loading rate causing 100% immobilization was 50 mg/l. The No-Observed-Effect Level (NOEL) was 4.5 mg/l based on immobilization.

Chemical analyses of the test solutions showed (mg/l as naphthalene):

Treatment Level	Day 0 New	Day 2 Old
Control	ND	0.0057
0.1	ND	ND
0.36	0.0390	ND
0.9	0.050	ND
4.5	0.41	0.13
23	1.9	0.083
50	3.2	1.7

ND = not detected

Deviations were noted for:
1) test substance was not held under refrigerated storage for approximately 3 weeks
2) not all test chambers contained five daphnids; four replicates had six and one replicate had four
3) due to a calculation error, a loading rate of 0.36 mg/l was used instead of the intended 0.2 mg/l
4) test daphnids were added directly to the test vessels rather than into intermediate vessels prior to adding to the test vessels
5) the dilution water was not aerated prior to use.

Test condition : Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs,

an appropriate volume of test substance was added quantitatively via syringe to glass carboys holding dilution water. The glass carboys were stoppered then the mixtures stirred for 24 hours (10% vortex, approximately 1-2 cm). An equilibration study run prior to the test justified a 24-hour stirring period. After a 1-hour settling period, WAF solutions were siphoned from the bottom of the carboy into glass test vessels. Test vessels were 125-ml glass Erlenmeyer flasks that were completely filled (no headspace) with the WAF solutions. Each test vessel was stoppered with glass closures to minimize evaporation and/or volatilization. Four replicate test vessels were prepared for each treatment level. Nominal loading rates used in the test were 0 (control), 0.1, 0.36, 0.9, 4.5, 23, and 50 mg/l. No undissolved test substance was observed in any of the WAF solutions during the test.

Daphnid cultures were maintained at the testing laboratory in dilution water supplemented with vitamin B12 and selenium. They were fed daily a green alga (Selenastrum capricornutum) and a yeast/salmon starter/cereal leaves mixture. Daphnids used in the test were 24 hours old that were taken from 17-day old parents.

To start the test, individual daphnids were randomly selected and distributed one at a time to each test vessel until each replicate test vessel contained five daphnids. Organism loading was approximately 1 daphnid per 28 ml of solution.

Samples of the WAF solutions were collected on Days 0 and 2 for chemical analysis. Day 0 samples were the freshly-prepared WAFs, while the Day 2 samples were collected as a composite from all replicates of a treatment level. WAF samples were extracted with hexane and analyzed by Gas Chromatography using a Flame Ionization Detector (GC-FID) and a capillary column. Measured concentrations were presented in the report as mg/l as naphthalene.

The test was conducted in a temperature-controlled waterbath under a 16 hour/8 hour light/dark photoperiod. Daylight intensity ranged from 68 to 70 foot-candles. Measurements of fresh solutions (Day 0) for water quality parameters showed dissolved oxygen concentration ranged from 8.3 to 9.4 mg/l, pH ranged from 7.5 to 8.4 and temperature was a constant 21 °C. Measurements of old test solutions (Day 2) indicated dissolved oxygen concentration ranged from 7.0 to 8.5, pH ranged from 7.7 to 8.2, and temperature was a constant 20 °C.

Dilution water was a mixture of carbon filtered well water and water dialyzed by reverse osmosis. This water had an alkalinity of 63 - 70 mg $CaCO_3$/l, hardness of 170 - 180 mg $CaCO_3$/l, specific conductance of 380 µmhos, pH of 7.6 - 7.7, and a dissolved oxygen concentration of 8.8 - 10.2 mg/l during the testing period. Total organic carbon measured 1.3 ppm during the monthly screening.

Reliability : (1) valid without restriction

(44)

Type : Static renewal
Species : Daphnia magna (Crustacea)
Exposure period : 48 hour(s)
Unit : mg/l
Limit Test : No
Analytical monitoring : Yes

Method : OECD Guide-line 202
Year : 1995
GLP : Yes
Test substance : CAS No. 64741-81-0; kerosene (petroleum), hydrodesulfurized

Method : EL_{50} values calculated using the Trimmed Spearman-Karber and probit methods, NOEL determined using Dunnett's Test.
Remark : The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, measurements (most) at the nominal level of 6.8 mg/l and below were extrapolations outside the calibration range.
Result : At the end of the test, all daphnids in the control group were alive and normal.

Daphnids exposed to the 0.1 mg/l WAF solution had one (5%) immobile daphnid at the end of the test with the remaining daphnids normal. Daphnids exposed to the 0.3 mg/l WAF solution had 15% immobilization, while daphnids exposed to the 1.4 mg/l WAF solution had 50% immobilization. Surviving daphnids of those two groups appeared normal. All daphnids exposed to 6.8 and 34 mg/l WAF solutions were immobile by Day 2 and Day 1, respectively, of the test.

The EL_{50} values with confidence intervals were:

Exposure Time, hours	EL_{50}, mg/l	Confidence Interval, mg/l
24	4.6	3.4 - 6.3
48	1.4	1.0 - 2.0

The maximum nominal loading causing no immobilization was not determined because all test levels had at least one immobile daphnid. The minimum nominal loading rate causing 100% immobilization was 6.8 mg/l. The No-Observed-Effect Level (NOEL) was 0.3 mg/l based on immobilization.

Chemical analyses of the test solutions showed (mg/l as naphthalene):

Treatment Level	Day 0 New	Day 2 Old
Control	0.0091	0.0065
0.1	0.0040	0.0008
0.3	0.047	0.0031
1.4	0.13	0.094
6.8	0.121	0.79
34	4.6	3.52

1 Report suggests material may have been lost during sampling.
2 Sample taken on Day 1 due to complete mortality.

Deviations were noted for:
1) test substance was not held under refrigerated storage for approximately 3 weeks
2) the test temperature deviated 0.12 °C for approximately seven minutes during the test
3) temperature measurements of the old solutions were not taken on Day 2
4) the dilution water was not aerated prior to the test
5) the definitive test loading rates did not follow a contiguous geometric series.

Test condition : Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs, an appropriate volume of test substance was added quantitatively via syringe to glass carboys holding dilution water. The glass carboys were stoppered then the mixtures stirred for 24 hours (10% vortex, approximately 1-2 cm). An equilibration study run prior to the test justified a 24-hour stirring period. After a 1-hour settling period, WAF solutions were siphoned from the bottom of the carboy into glass test vessels. Test vessels were 125-ml glass Erlenmeyer flasks that were completely filled (no headspace) with the WAF solutions. Each test vessel was stoppered with glass closures to minimize evaporation and/or volatilization. Four replicate test vessels were prepared for each treatment level. Nominal loading rates used in the test were 0 (control), 0.1, 0.3, 1.4, 6.8, and 34 mg/l. No undissolved test substance was observed in any of the WAF solutions during the test.

Daphnid cultures were maintained at the testing laboratory in dilution water supplemented with vitamin B12 and selenium. They were fed daily a green alga (Selenastrum capricornutum) and a yeast/salmon starter/cereal leaves mixture. Daphnids used in the test were 24 hours old that were taken from 13 and 15-day old parents.

To start the test, individual daphnids were randomly selected and distributed one at a time to intermediate containers until each container held five daphnids. Daphnids were then transferred to their respective test vessel. Organism loading was approximately 1 daphnid per 28 ml of solution.

Samples of the WAF solutions were collected on Days 0 and 2 for chemical analysis. Day 0 samples were the freshly-prepared WAFs, while the Day 2 samples were collected as a composite from all replicates of a treatment level. WAF samples were extracted with hexane and analyzed by Gas Chromatography using a Flame Ionization Detector (GC-FID) and a capillary column. Measured concentrations were presented in the report as mg/l as naphthalene.

The test was conducted in a temperature-controlled waterbath under a 16 hour/8 hour light/dark photoperiod. Daylight intensity ranged from 67 to 68 foot-candles. Measurements of fresh solutions (Day 0) for water quality parameters showed dissolved oxygen concentration ranged from 8.0 to 9.5 mg/l, pH ranged from 8.0 to 8.7 and temperature was a constant 20 °C. Measurements of old test solutions (Day 2) indicated dissolved oxygen concentration ranged from 7.2 to 8.1 and pH ranged from 7.6 to 8.6. The temperature of the old solutions was not measured.

Dilution water was a mixture of carbon filtered well water and water dialyzed by reverse osmosis. This water had an alkalinity of 64 - 70 mg $CaCO_3$/l, hardness of 170 - 174 mg $CaCO_3$/l, specific conductance of 380 - 420 µmhos, pH of 7.6, and a dissolved oxygen concentration of 8.5 - 8.8 mg/l during the testing period. Total organic carbon measured 1.3 ppm during the monthly screening.

Reliability : (1) valid without restriction

(45)

Type	:	Static
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit Test	:	No
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 202
Year	:	1993
GLP	:	Yes
Test substance	:	CAS No. 64742-81-0; Kerosene, hydrotreated
Method	:	Visual inspection
Result	:	48-hr EL_{50} lies between 40 and 89 mg/l WAF, based on nominal loading rates. Numbers of immobilized daphnids after 48 hrs were 0, 0, 0, 0, 14, and 20 in the 0, 8.3, 18, 40, 89, and 200 mg/l treatments. No excursions from protocol were noted. Analytical method used was gas chromatography-mass spectrometry. Mean reduction in the concentration of dissolved components of kerosene during the test was 31%, 22%, and 34% in the 8.3, 40, and 200 mg/l WAFs. The sample collected at 0 hr for the 89 mg/l WAF was lost during analysis, thus, the percent reduction could not be determined for that treatment.
Test condition	:	Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 8.3, 18, 40, 89, and 200 mg/l. Control and dilution water was reconstituted hard water prepared by adding salts to reverse osmosis filtered water following EPA guidelines (hardness 180 mg/l as $CaCO_3$). Test substance was mixed in dilution water for 23 hrs in sealed vessels with minimal headspace. Mixing time was determined in an equilibration study in which the test substance concentration in the aqueous phase of the WAFs was monitored by GC-MS. Mixtures were allowed to settle 1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 150-ml Erlenmeyer flasks completely filled with test solution and contained 10 daphnids per vessel. Test daphnids were <24 hrs old and collected from cultures supplied by the testing laboratory that have been aged between 14 and 28 days. Two replicates per treatment and control were used. Test temperature was 18 - 19 °C. Photoperiod was 16 hrs light and 8 hrs dark. Dissolved oxygen was >60% saturation (9.2 to 9.7 mg/l). pH was 7.6 - 8.2. Samples were collected at the beginning and end of the test to monitor the concentration of dissolved components of kerosene in the test solutions.
Reliability	:	(1) valid without restriction

(69)

Type	:	Static renewal
Species	:	Daphnia magna (Crustacea)
Exposure period	:	48 hour(s)
Unit	:	mg/l
Limit Test	:	No
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 202
Year	:	1995
GLP	:	Yes

Test substance : CAS No. 101316-80-7; solvent naphtha (petroleum), hydrocracked heavy aromatic

Method : Statistical method:
EL_{50} values calculated using the probit method, NOEL determined using Duncan's Multiple Range Test.

Remark : The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, the measurements at the nominal level of 1.4 mg/l and below were extrapolations outside the calibration range.

Result : At the end of the test, two individuals (10%) from the control group were immobile, all other daphnids of the control group appeared normal. Daphnids exposed to the 0.1 mg/l WAF solution had one (5%) immobile daphnid at the end of the test, while all daphnids exposed to the 0.3 mg/l WAF solution were normal. Exposure to the 1.4 mg/l WAF solution caused 35% immobility with the surviving daphnids all normal. By the end of the test, all daphnids exposed to 6.8 and 34 mg/l WAF solutions were immobile.

The EL_{50} values with confidence intervals were:

Exposure Time, hours	EL_{50}, mg/l	Confidence Interval, mg/l
24	28	22 - 35
48	1.9	1.3 - 4.3

The maximum nominal loading causing no immobilization was 0.3 mg/l. The minimum nominal loading rate causing 100% immobilization was 6.8 mg/l. The No-Observed-Effect Level (NOEL) was 0.3 mg/l based on immobilization.

Chemical analyses of the test solutions showed (mg/l as naphthalene):

Treatment Level	Day 0 New	Day 2 Old
Control	0.029	0.0018
0.1	0.141	0.151
0.3	0.041	0.050
1.4	0.035	0.030
6.8	0.91	0.94
34	2.4	2.4

1 Report suggests high values due to contamination.

Deviations were noted for:

1) On Day 1 of the test biological observations were made outside the ± 1-hour limit
2) temperature measurements of the old solutions were not taken on Day 2
3) the test temperature deviated by 0.1 °C beyond the ± 1 °C limit for the test for approximately 34 hours
4) test substance was not held under refrigerated storage for approximately 3 weeks
5) the definitive test loading rates did not follow a contiguous geometric series.

Test condition : Daily renewal of the test media ensured that test material levels were maintained at the exposure concentrations. Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs,

an appropriate volume of test substance was added quantitatively via syringe to glass carboys holding dilution water. The glass carboys were stoppered then the mixtures stirred for 24 hours (10% vortex, approximately 1-2 cm). An equilibration study run prior to the test justified a 24-hour stirring period. After a 1-hour settling period, WAF solutions were siphoned from the bottom of the carboy into glass test vessels. Test vessels were 125-ml glass Erlenmeyer flasks that were completely filled (no headspace) with the WAF solutions. Each test vessel was stoppered with glass closures to minimize evaporation and/or volatilization. Four replicate test vessels were prepared for each treatment level. Nominal loading rates used in the test were 0 (control), 0.1, 0.3, 1.4, 6.8, and 34 mg/l. No undissolved test substance was observed in any of the WAF solutions during the test.

Daphnid cultures were maintained at the testing laboratory in dilution water supplemented with vitamin B12 and selenium. They were fed daily a green alga (Selenastrum capricornutum) and a yeast/salmon starter/cereal leaves mixture. Daphnids used in the test were 24 hours old that were taken from 13-day old parents.

To start the test, individual daphnids were randomly selected and distributed one at a time to intermediate containers until each container held five daphnids. Daphnids were then transferred to their respective test vessel. Organism loading was approximately 1 daphnid per 28 ml of solution.

Samples of the WAF solutions were collected on Days 0 and 2 for chemical analysis. Day 0 samples were the freshly-prepared WAFs, while the Day 2 samples were collected as a composite from all replicates of a treatment level. WAF samples were extracted with hexane and analyzed by Gas Chromatography using a Flame Ionization Detector (GC-FID) and a capillary column. Measured concentrations were presented in the report as mg/l as naphthalene.

The test was conducted in a temperature-controlled waterbath under a 16 hour/8 hour light/dark photoperiod. Daylight intensity ranged from 66 to 68 foot-candles. Measurements of fresh solutions (Day 0) for water quality parameters showed dissolved oxygen concentration ranged from 8.4 to 9.5 mg/l, pH ranged from 7.8 to 8.4 and temperature was a constant 20 °C. Measurements of old test solutions (Day 2) indicated dissolved oxygen concentration ranged from 7.8 to 8.2 and pH ranged from 7.6 to 8.4. The temperature of the old solutions was not measured.

Dilution water was a mixture of carbon filtered well water and water dialyzed by reverse osmosis. This water had an alkalinity of 64 - 70 mg $CaCO_3$/l, hardness of 170 - 174 mg $CaCO_3$/l, specific conductance of 380 - 420 µmhos, pH of 7.6, and a dissolved oxygen concentration of 8.5 - 8.8 mg/l during the testing period. Total organic carbon measured 1.3 ppm during the monthly screening.

Reliability : (1) valid without restriction

(46)

4.3 TOXICITY TO AQUATIC PLANTS E.G. ALGAE

Species	:	Selenastrum capricornutum (Algae)
Endpoint	:	Growth rate
Exposure period	:	96 hour(s)
Unit	:	mg/l
Limit test	:	No
Analytical monitoring	:	Yes
Method	:	OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year	:	1995
GLP	:	Yes
Test substance	:	CAS No. 64742-81-0; kerosene (petroleum), hydrodeulfurized
Method	:	EL_{50} values calculated by the inverse interpolation method of Snedecor and Cochran (1989); NOEL value determined using Analysis of Variance.
Remark	:	The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, the measurements at the nominal level of 4.0 mg/l and below were extrapolations outside the calibration range.
Result	:	The percent inhibition values for average specific growth rate (ASGR) and area under the growth curve (AUGC) at 72 and 96 hours for each WAF loading level were:

Loading	**% Inhibition Relative to Controls**			
Rate	**ASGR**		**AUGC**	
mg/l	**72 H**	**96 H**	**72 H**	**96 H**
0.4	-4.8	0	-41	-20
4.0	6.8	7.5	13	21
20	45	48	75	87
45	96	100	98	99
100	100	100	99	99

Note: negative values indicate a stimulatory effect.
The EL_{50} values with confidence intervals were:

	EL_{50}, mg/l	**95% Confidence Limits**
ASGR		
0 - 72 h	8.3	could not calculate
0 - 96 h	6.2	could not calculate
AUGC		
0 - 72 h	15	0 - 52
0 - 96 h	11	0 - 42

The No-Observed-Effect Levels determined for ASGR and AUGC were:

ASGR NOEL	72 h	=	4.0 mg/l
	96 h	=	0.4 mg/l
AUGC NOEL	72 h	=	4.0 mg/l
	96 h	=	0.4 mg/l

Chemical analyses of the test solutions showed:

Treatment	**Measured Concentration (mg/l as naphthalene)**	
Level	**Day 0**	**Day 4**
Control	0.015	Not detedcted
0.4 mg/l	0.0083	0.020
4.0	0.23	0.15
20	0.86	0.89
45	3.9	2.7
100	5.4	4.6

Deviations were noted for:
1) test substance was not held under refrigerated storage for approximately 3 weeks
2) loading rates assigned for the test were not in a contiguous geometric series.

Test condition : Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs, an appropriate volume of test substance was added quantitatively to 1.8 liters algal nutrient media in a 2-liter glass aspirator bottle. The bottles were stoppered then stirred (10% vortex, approximately 1 cm) for approximately 24 hours (an equilibration study conducted prior to the test justified a 24-hour stirring period). After a 1-hour settling period, the WAF solutions were removed through an outlet at the bottom of the bottle. A portion of each WAF solution was used to condition the replicate test vessels by placing 25 ml of solution per flask and allowing the solutions to sit for approximately 5 minutes. The flasks then were swirled and the solution discarded. Each replicate test vessel was filled with 140 ml of WAF, inoculated with algae and immediately closed with a ground glass stopper. Test vessels were 125-ml glass Erlenmeyer flasks, and six replicate vessels were used in the control group, while three replicate vessels were used for each WAF solution. Nominal loading rates used in the test were 0 (control), 0.4, 4.0, 20, 45, and 100 mg/l. No undissolved test substance was observed in the test vessels during the test.

Nutrient medium was prepared according to the formulation described by Miller et al. 1978 (EPA-600/9-78-018) with additional sodium bicarbonate* (NaHCO3 at 100 mg/l) added as a source of carbon in the sealed test chambers. The nutrient medium was prepared with distilled water and reagent grade chemicals at the following concentrations:

Compound	Concentration mg/l	Compound	Concentration µg/l
$NaNO_3$	25.5	H_3BO_3	185.52
$MgCl_2.6H_2O$	12.164	$MnCl_2.4H_2O$	415.38
$CaCl_2.2H_2O$	4.41	$ZnCl_2$	3.27
$MgSO_4.7H_2O$	14.7	$CoCl_2.6H_2O$	1.428
K_2HPO_4	1.044	$CuCl_2.2H_2O$	0.012
$NaHCO_3$	*see above	$Na2MoO_4.2H_2O$	7.26
		$FeCl_3.6H_2O$	159.76
		$Na_2EDTA.2H_2O$	300.0

S. capricornutum cultures were maintained by the testing laboratory at a temperature of 24 ± 2 °C under constant illumination of 4300 10% lux provided by cool-while fluorescent bulbs. The source of the culture was the Department of Botany, University of Texas (initial strain #1648). The algal inoculum used to start the test originated from 5-day old stock cultures in log phase growth.

To start the test, the different WAF and control solutions were placed in their respective number of replicate flasks and inoculated with S. capricornutum to an initial cell density of 1.0×10^3 cells/ml. The flasks were sealed and randomly positioned on a shaker table. All flasks were incubated for four days under constant illumination (4300 to 4400 Lux), continuous shaking at 125 rpm, and at a mean temperature of 23.6 °C.

Cell density was determined for each replicate flask at 24, 48, 72, and 96

hours. This was done by taking 3.5 ml of solution from each flasks and measuring fluorescence on a Turner Filter fluorometer and converting fluorescence readings to algal cell densities using a regression formula developed through cell counts. In order to maintain no headspace in the test flasks, the 3.5 ml aliquot of solution removed for analyses was replaced with 3.5 ml of spare WAF. The spare WAF samples were stored in closed vessels, and a new vessel was opened for each replacement interval. The replacement of 3.5 ml of WAF diluted the cell densities by approximately 2.5% on Days 2-4. The overall affect of this was considered not significant.

The pH of the control and test solutions were taken at 0 and 96 hours. The pH of the solutions ranged from 7.4 to 7.5 at the beginning of the test and 7.6 to 9.8 at the end of the test.

The EL_{50} values were determined on the percent inhibition relative to the control values for average specific growth rate and area under the growth curve. The specific growth rates for each treatment were determined by calculating the slope of the regression line of ln(cell density) versus time. The area under the growth curves was calculated in accordance with the equations in OECD Guideline No. 201

Reliability : (1) valid without restriction

(51)

Species : Selenastrum capricornutum (Algae)
Endpoint :
Exposure period : 72 hour(s)
Unit : mg/l
Limit test : no
Analytical monitoring : yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1993
GLP : Yes
Test substance : CAS No. 64742-81-0, Kerosene, hydrotreated

Method : EL_{50} values determined by visual inspection. Williams test used to determine NOELs.

Result :

Based on nominal loading rates: 72-hr EL_{50} (biomass) lies between 10 and 30 mg/l
72-hr EL_{50} (growth rate) lies between 10 and 30 mg/l
72-hr NOEL (biomass) = 1 mg/l
72-hr NOEL (growth rate) = 10mg/l.

Nominal Conc. (mg/l)	72 h % Inhibition	72 h Mean Cell Conc. (million cells/ml)
Control	n/a	0.15
0.3	6.7	0.14
1.0	20	0.12
3.0	20	0.12
10	33	0.10
30	91	0.013

n/a - Not applicable
The pH increased by more than one unit during the test as a result of good culture growth and could not be avoided. This deviation was not sufficient to invalidate the study.
Analytical method used was gas chromatography-mass spectrometry.
Mean reduction in the concentration of dissolved components of kerosene

during the test was 50%, 0%, 50%, and 19% in the 1, 3, 10, and 30 mg/l WAFs.

Test condition : Individual treatment concentrations were prepared as water accommodated fractions (WAF). Nominal loading rates in the definitive test were 0, 0.3, 1, 3, 10 and 30 mg/l. Control and dilution water was algal nutrient medium prepared according to EPA guidelines except that boric acid was present at 105 g/l and sodium bicarbonate at 50 mg/l. Test substance was mixed with dilution water for 20 hrs, and the mixture was allowed to settle for 1 hr prior to drawing off the aqueous phase for testing. Test vessels were sealed 300 ml Erlenmeyer flasks completely filled with test solution. There were four flasks for each treatment and seven control flasks. Three of the four treatment and six of the seven control flasks were inoculated with algal cells to yield an initial concentration of 5000 cells/ml. Algal cells were obtained from laboratory cultures that were originally derived from a strain from American Type Culture Collection (ATCC 22662). Uninoculated flasks were used to determine particle counts without algal cells using a Coulter Multisizer. Flasks were incubated in a cooled orbital (100 cycles/min) incubator. Biomass was calculated as area under the growth curve. Test temperature was 24 - 25 °C. Lighting was continuous at ~3000 lux. The pH ranged from 7.3 - 7.5 at test initiation and 8.6 - 9.4 at test termination. Samples were collected at the beginning and end of the test to monitor the concentration of dissolved components of kerosene in the test solutions.

Reliability : (1) valid without restriction

(71)

Species : Selenastrum capricornutum (Algae)
Exposure period : 96 hour(s)
Unit : mg/l
Limit test : No
Analytical monitoring : Yes
Method : OECD Guide-line 201 "Algae, Growth Inhibition Test"
Year : 1995
GLP : yes
Test substance : CAS No. 101316-80-7; solvent naphtha (petroleum), hydrocracked heavy aromatic

Method : Statistical method
EL_{50} values calculated by the inverse interpolation method of Snedecor and Cochran (1989); NOEL value determined using Duncan's Multiple Range Test.

Remark : The report did not specify the minimum level of detection for the analytical measurements, but the lowest calibration standard was 0.66 mg/l. Given this, the measurements at the nominal level of 50 mg/l and below were extrapolations outside the calibration range.

Result : The percent inhibition values for average specific growth rate (ASGR) and area under the growth curve (AUGC) at 72 and 96 hours for each WAF loading level were:

Loading Rate	% Inhibition Relative to Controls			
	ASGR		AUGC	
mg/l	72 H	96 H	72 H	96 H
0.2	1.4	1.4	8.0	10
0.8	-3.2	-1.9	-13	-9.4
6.2	-4.4	-1.5	-34	-25
12	58	68	84	94
50	92	97	98	100

Note: negative values indicate a stimulatory effect.

The EL_{50} values with confidence intervals were:

	EL_{50}, mg/l	95% Confidence Limits
ASGR		
0 - 72 h	6.7	0.4 - 896
0 - 96 h	5.0	0.5 - 131
AUGC		
0 - 72 h	12	could not calculate
0 - 96 h	5.9	could not calculate

The No-Observed-Effect Levels determined for ASGR and AUGC were:

ASGR NOEL 72 and 96 hours = 6.2 mg/l
AUGC NOEL 72 and 96 hours = 12 mg/l

Chemical analyses of the test solutions showed:

Treatment Level	Measured Concentration (mg/l as naphthalene) Day 0	Day 4
Control	0.015	ND
0.2 mg/l	ND	ND
0.8	ND	0.021
6.2	0.0059	ND
12	0.51	0.44
50	2.0	1.8

Note: the authors state that the 6.2 mg/l test level may have been dosed at 3.1 mg/l, but this could not be concluded.

Deviations were noted for:
1) test substance was not held under refrigerated storage for approximately 3 weeks
2) loading rates assigned for the test were not in a contiguous geometric series.

Test condition : Test solutions were prepared as water accommodated fractions (WAFs) with each treatment level prepared independently of other treatment levels. To prepare the WAFs, an appropriate volume of test substance was added quantitatively to 1.8 liters algal nutrient media in a 2-liter glass aspirator bottle. The bottles were stoppered then stirred (10% vortex, approximately 1 cm) for approximately 24 hours (an equilibration study conducted prior to the test justified a 24-hour stirring period). After a 1-hour settling period, the WAF solutions were removed through an outlet at the bottom of the bottle. A portion of each WAF solution was used to condition the replicate test vessels by placing 25 ml of solution per flask and allowing the solutions to sit for approximately 5 minutes. The flasks then were swirled and the solution discarded. Each replicate test vessel was filled with 140 ml of WAF, inoculated with algae and immediately closed with a ground glass stopper. Test vessels were 125-ml glass Erlenmeyer flasks, and six replicate vessels were used in the control group, while three replicate vessels were used for each WAF solution. Nominal loading rates used in the test were 0 (control), 0.2, 0.8, 6.2, 12, and 50 mg/l. No undissolved test substance was observed in the test vessels during the test.

Nutrient medium was prepared according to the formulation described by Miller et al. 1978 (EPA-600/9-78-018) with additional sodium bicarbonate* ($NaHCO_3$ at 100 mg/l) added as a source of carbon in the sealed test chambers. The nutrient medium was prepared with distilled water and reagent grade chemicals at the following concentrations:

Compound	Concentration, mg/l	Compound	Concentration µg/l
$NaNO_3$	25.5	H_3BO_3	185.52
$MgCl_2.6H_2O$	12.164	$MnCl_2.4H_2O$	415.38
$CaCl_2.2H_2O$	4.41	$ZnCl_2$	3.27
$MgSO_4.7H_2O$	14.7	$CoCl_2.6H_2O$	1.428
K_2HPO4	1.044	$CuCl_2.2H_2O$	0.012
$NaHCO_3$	*see above	$Na_2MoO_4.2H_2O$	7.26
		$FeCl_3.6H_2O$	159.76
		$Na_2EDTA.2H_2O$	300.0

S. capricornutum cultures were maintained by the testing laboratory at a temperature of 24±2 °C under constant illumination of 4300 10% lux provided by cool-while fluorescent bulbs. The source of the culture was the Department of Botany, University of Texas (initial strain #1648). The algal inoculum used to start the test originated from 5-day old stock cultures in log phase growth.

To start the test, the different WAF and control solutions were placed in their respective number of replicate flasks and inoculated with S. capricornutum to an initial cell density of 1.0×10^3 cells/ml. The flasks were sealed and randomly positioned on a shaker table. All flasks were incubated for four days under constant illumination (4200 to 4400 Lux), continuous shaking at 125 rpm, and at a mean temperature of 23.6 °C.

Cell density was determined for each replicate flask at 24, 48, 72, and 96 hours. This was done by taking 3.5 ml of solution from each flasks and measuring fluorescence on a Turner Filter fluorometer and converting fluorescence readings to algal cell densities using a regression formula developed through cell counts. In order to maintain no headspace in the test flasks, the 3.5 ml aliquot of solution removed for analyses was replaced with 3.5 ml of spare WAF. The spare WAF samples were stored in closed vessels, and a new vessel was opened for each replacement interval. The replacement of 3.5 ml of WAF diluted the cell densities by approximately 2.5% on Days 2-4. The overall affect of this was considered not significant.

The pH of the control and test solutions were taken at 0 and 96 hours. The pH of the solutions ranged from 7.5 to 7.6 at the beginning of the test and 7.7 to 9.9 at the end of the test.

The EL_{50} values were determined on the percent inhibition relative to the control values for average specific growth rate and area under the growth curve. The specific growth rates for each treatment were determined by calculating the slope of the regression line of ln(cell density) versus time. The area under the growth curves was calculated in accordance with the equations in OECD Guideline No. 201.

Reliability : (1) valid without restriction

(49)

5.1.1 ACUTE ORAL TOXICITY

Type	:	LD_{50}
Value	:	> 5000 mg/kg bw
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	Male/female
Number of animals	:	5
Vehicle	:	Undiluted
Doses	:	Single dose of 5 g/kg bwt
Year	:	1985
GLP	:	Yes
Test substance	:	Straight run kerosene, sample API 83-09 (See section 1.1.1.)
Method	:	The test material was administered by oral gavage as a single dose of 5 g/kg to five male and five female Sprague-Dawley rats. Food was withheld overnight before administration of test material whilst water was available ad libitum. The animals were observed for clinical signs and mortality at hourly intervals for the first six hours after dosing and twice daily thereafter for 14 days. Body weights were recorded before fasting, just prior to administration of test material and 7 and 14 days after test material administration. At the end of the study, all animals were killed and underwent a gross necropsy. Any abnormalities were recorded.
Result	:	Animals gained weight following administration of the test material. Clinical signs observed included: hypoactivity, ataxia, prostration, soft stool, lacrimation, yellow-stained abdomen and/or urogenital region and hair loss on abdomen and/or urogenital region. There were no mortalities during the study. There were no gross abnormalities at necropsy.
Reliability	:	(1) valid without restriction

(16)

Type	:	LD_{50}
Result	:	Two additional acute oral toxicity studies have been reported for substances in this group. The results were as follows:

Sample	**LD_{50}**	**Report reference**
Hydrodesulfurized kerosene API 81-07		
	> 5g/kg	API 30-31986
Jet Fuel A	>20 g/kg	API 27-32815

(8) (10)

5.1.2 ACUTE INHALATION TOXICITY

Type	:	LC_{50}
Value	:	> 5 mg/l
Species	:	Rat
Strain	:	Sprague-Dawley
Sex	:	male/female
Number of animals	:	5
Vehicle	:	Air

Doses	:	5.28 ±0.42 mg/l
Exposure time	:	4 hour(s)
Year	:	1987
GLP	:	Yes
Test substance	:	Straight run kerosene, sample API 83-09 (See section 1.1.1.)
Method	:	Five male and five female Sprague-Dawley rats were exposed to approximately 5 mg/l of test material as a single four-hour whole body inhalation exposure. After the exposure the rats were kept for a 14 day observation period. Surviving animals at 14 days were sacrificed and subjected to a gross post-mortem examination. Records were made of any observed gross abnormalities. The lungs of all animals were preserved in formalin, sectioned and stained and then subjected to microscopic examination. Since no animals died following the exposure, no further exposures were conducted.
Result	:	All except one animal had normal growth rates throughout the study. The one exception on day 8 had a body weight less than its starting body weight but by the end of the study normal growth had resumed. Decreased activity was exhibited by all animals during the exposure. Otherwise there were no treatment-related clinical signs of toxicity. No macroscopic lesions were observed in any animal at post-mortem and no microscopic changes were observed in any lung section examined.
Test condition	:	Exposure of the animals was conducted in a 250 liter stainless steel and glass chamber. The test material was introduced at a rate of 0.34 to 0.51 ml/minute into the top of a vertical counter current column that was packed with steel mesh and heated to approximately 50 °C. Pre-warmed nitrogen gas was introduced at the bottom of the column at a rate of 7 liters/minute. The nitrogen and test material vapors were then mixed with air and introduced into the exposure chamber. The test atmosphere was analyzed by IR every 15 minutes throughout the exposure. During each hour of the exposure, the test atmosphere was analyzed gravimetrically and visually (by flashlight) for aerosols of the test material. Test material consumption was determined by weighing the test material container before and after exposure. The quantity consumed was divided by the total airflow and this yielded the nominal exposure concentration. The mean exposure concentrations were: Nominal concentration 5.74 mg/l Analytical concentration 5.28 ±0.42 mg/l Gravimetric samples, collected on membrane filters, and aerosol checks with a flashlight showed some aerosol in the chamber. The nominal to analytical ratio and the gravimetric results both suggest the level of aerosol compared to level of vapor was insignificant in the exposure.
Reliability	:	(1) valid without restriction

(21)

Type : LC_{50}
Exposure time :

Result : Additional acute inhalation toxicity studies have also been reported for substances in this group.

Sample (Species)	LC_{50}	Report reference
Hydrodesulfurized kerosene API 81-07		
(Rat, 4-hour)	> 5.2 mg/l	API 30-32855
Deodorized kerosene		
(Rat, 8-hour)	No mortalities when exposed to saturated vapor	Carpenter et al
(Cat, 6-hour)	>6.4 mg/l	Carpenter et al (12) (34) (43)

5.1.3 ACUTE DERMAL TOXICITY

Type : LD_{50}
Value : > 2000 mg/kg bw
Species : Rabbit
Strain : New Zealand white
Sex : Male/female
Number of animals : 2
Vehicle : Undiluted
Doses : 2 g/kg
Year : 1985
GLP : Yes
Test substance : Straight run kerosene, sample API 83-09 (See section 1.1.1.)

Method : Undiluted test material was applied to the shorn dorsal skin of each of two male and two female rabbits. One rabbit of each sex had abraded skin, the other had intact skin. The area of application was wrapped with gauze and overwrapped with an occlussive covering. 24 hours later, the covering was removed and the skin was wiped with wet disposable towels to remove any residual test material.
The rabbits were observed for clinical signs and mortality for the first six hours of dosing, then daily for dermal irritation and twice daily for clinical signs of toxicity and mortality for 14 days. Body weights were recorded just prior to and again 7 days and 14 days after administration of test material.
At study termination all animals were killed and subjected to a gross necropsy examination when any abnormalities were recorded.

Result : Clinical signs observed during the study included hypoactivity and diarrhea.
Dermal irritation ranged from
- slight to severe for erythema and edema
- slight to marked for atonia, desquamation and fissuring
- slight to moderate for coriaceousness.

Other dermal irritation observed included subcutaneous hemorrhage, blanching and scab formation.
No animals died during the study.

One of the rabbits (male abraded skin) weighed slightly less at the end of the study than at the beginning. All other rabbits had gained a small amount of body weight by the end of the study.

Reliability : (1) valid without restriction

(16)

Type : LD_{50}

Result : Two additional acute dermal toxicity studies have been reported for substances in this group. The results were as follows:

Sample	LD_{50}	Report reference
Hydrodesulfurized kerosene API 81-07		
	> 2g/kg	API 30-31986
Jet Fuel A	>4 g/kg	API 27-32815

(8) (10)

5.2.1 SKIN IRRITATION

Species : Rabbit
Concentration : Undiluted
Exposure : Occlusive
Exposure time : 24 hour(s)
Number of animals : 6
Vehicle : None
PDII : 5.5
Year : 1985
GLP : Yes
Test substance : Straight run kerosene, sample API 83-09 (See section 1.1.1.)

Method : Six rabbits were used in this study.
Approximately 24 hours before the study the hair was clipped from the back and flanks of each animal. Just prior to the test material administration, abraded areas were prepared; these were not sufficiently deep to cause bleeding. 0.5 ml of test material was applied to two areas of skin on each animal (abraded and intact skin). The treated areas were each covered with a gauze which was secured with paper tape and then covered with an occlusive dressing. After 24 hours exposure, the patches were removed and residual test material was removed from the skin by gentle wiping with a wet paper tape. The degree of erythema and edema was recorded using the Draize scale. A second assessment of skin reaction was made at 72 hours. Because irritation was still present at 72 hours, further evaluations of skin irritation were made at 96 hours, 7 days and 14 days post exposure. Body weights were recorded just prior to the application of the test material and again at weekly intervals throughout the study.

Result : The results are given in the following table.

Observation time	Erythema		Edema		Total score
	Intact	Abraded	Intact	Abraded	
24 hrs	2.5	2.8	2.8	3.2	5.7
72 hrs	2.8	3.0	2.3	2.2	5.2
96 hrs	2.8	3.0	2.7	2.5	5.5
7 days	2.3	2.3	2.0	2.2	4.4
14 days	0.3	0.7	0.0	0.0	0.5

Primary dermal irritation Index= 5.5

The primary dermal irritation index is the sum of the irritation scores for 24 and 72 hours divided by 2 and rounded to the nearest tenth.
The test material produced moderate to severe levels of irritation.
Blanching was seen within the test sites of two animals at 24 and 72 hours and of three animals at 96 hours. Subcutaneous hemorrhages were also observed in two animals at 96 hours.
There were no signs of systemic toxicity.

Reliability : (1) valid without restriction

(16)

Species : Rabbit

Result : A further six skin irritation studies have been reported for this group of substances. The results are:

Material	Skin irritation	Reference
Hydrodesulphurized kerosene API 81-09		
	Mild-moderate (24 hr)	API 30-31986
Odorless kerosene		
	Mild (4 hour)	Shell SBER 91-001
Kerosene SG	Mild (4 hour)	Shell SBER 91-003
Hydrocracked kerosene		
	Mild (4 hour)	Shell SBER 91-004
Jet A-1	Mild (4 hour)	Shell 91-005
Jet fuel A	Moderate-severe (24 hr)	API 27-32815

(8) (10) (65) (66) (67) (68)

5.2.2 EYE IRRITATION

Species : Rabbit
Concentration : Undiluted
Dose : 0.1 ml
Comment : One group of animals, eyes rinsed after 30 seconds
Vehicle : None
Year : 1985
GLP : Yes
Test substance : Straight run kerosene, sample API 83-09 (See section 1.1.1.)

Method : 0.1 ml undiluted test material was dripped onto the corneal surface of one eye of each of nine rabbits. The upper and lower eyelids were held together for one second to prevent loss of test material. 20-30 seconds after application of test material the eyes of three rabbits were flushed for 1 minute with lukewarm water. The other six rabbits did not receive any further treatment.
The eyes of each rabbit were examined 1, 24, 48 and 72 hours and seven days after application of test material. Sodium fluorescein was as an aid to reveal possible corneal injury. Grading and scoring of ocular lesions was performed according to the Draize scale. Body weights were recorded just before treatment and again at the end of the study.

Result : A pain response was elicited in one animal following instillation of test material. No corneal or iridial irritation was seen during the study. All irritation had cleared by the 24 hour observation time.
No systemic toxicity was seen during the study and body weights were unaffected by treatment. The primary irritation score recorded at the 1 hour observation period was:

	Unwashed eyes	Washed eyes
	6 rabbits	3 rabbits
1 hour	0.7	2.0

The scores for all other observation times were zero.
The primary irritation score is the total eye irritation score for all animals, divided by the number of animals in each group.

Reliability : (1) valid without restriction

(16)

Species : Rabbit

Result : Two other eye irritation studies have been reported.

For these studies on hydrodesulphurized kerosene (API Report No. 30-31986) and jet fuel (API report 27-32815), the method of scoring ocular lesions and calculating primary eye irritation index was exactly comparable to the study described above on straight run kerosene. The results for these two materials are as follows:

Test material	Primary eye irritation index	
	Unwashed eyes	Washed eyes
Hydrodesulphurized kerosene		
1 hour	3.0	2.7
24 hours	0.3	0.7
48 hours	0.3	0
72 hours	0	0
7 days	0	0
Jet fuel		
24 hour	3.33	1.33
48 hour	1.0	1.33
72 hour	1.0	1.33
7 day	0	0

(8) (10) (42)

5.3 SENSITIZATION

Type : Buehler Test
Species : Guinea pig
Concentration : 1st: Induction 75 % occlusive epicutaneous
2nd: Challenge 10 % occlusive epicutaneous
Number of animals : 10
Vehicle : Paraffin oil
Result : Not sensitizing
Year : 1985
GLP : Yes
Test substance : Straight run kerosene, sample API 83-09 (See section 1.1.1.)

Method : 0.4 ml undiluted test material was applied under an occlusive dressing to the shaved skin of 10 animals. Six hours after application, the dressing was removed and the skin wiped to remove residues of test material. The animals received one application each week for 3 weeks. The same skin site was used for the first two applications but due to the severe irritation that had occurred in the test and positive control groups a different site was used for the third sensitizing application.
2 weeks following the third application a challenge dose (0.4 ml of a 1%

solution in paraffin oil) was applied in the same manner as the sensitizing doses. A previously untreated site was used for the challenge application. The application sites for sensitizing and challenge doses were scored for erythema and edema 24 and 48 hours after patch removal. To assist in the scoring of the response to the final challenge dose the test site was depilated 3 hours prior to examination by using a commercially available depilatory cream.
Positive control (2,4-dinitrochlorobenzene), vehicle control and naive control groups were included in this study.

Concentrations of positive control were as follows:
Sensitizing doses: 0.4 ml of 0.3% w/v in 80% aqueous ethanol
Challenge dose: 0.4 ml of 0.1% w/v suspension in acetone

Result : The skin reactions after challenge applications were as follows:

test group	No dermal irritation in any animal
Naive control group	Very slight erythema in two animals, no reaction in the other eight animals
Vehicle control group	A very slight erythema in one animal, no reaction in the other nine animals
Positive control group	Very slight to severe irritation in all 20 animals. The reaction in 19 animals exceeded the highest reaction observed in the naive positive control animals
Naive positive control group	5 of 20 animals exhibited very slight eryrthema, the other 15 animals had no skin reaction.

Reliability : (1) valid without restriction

(16)

Type : Buehler Test

Result : Jet Fuel A was not a skin sensitizer in a Beuhler test (Report API 27-32815).
Hydrodesulfurized kerosene was not a skin sensitizer in a Beuhler test (Report API 31-31413).

(8) (13)

5.4 REPEATED DOSE TOXICITY

Type :
Species : Rabbit
Sex : Male/female
Strain : New Zealand white
Route of admin. : Dermal
Exposure period : 28 days
Frequency of treatm. : Three times weekly
Doses : 200, 1000 and 2000mg/kg/day
Control group : Yes, concurrent no treatment
Method :
Year : 1985
GLP : No
Test substance : Straight run kerosene, sample API 83-09 (See section 1.1.1.)

Method : Undiluted test material was applied to the shorn dorsal skin of each of five

male and five female rabbits at doses of 200, 1000 and 2000 mg/kg/day, three times weekly until 12 doses had been applied. Five rabbits of each sex served as sham treated controls. Dosing was carried out on alternate days.
Each treated site was covered with a gauze pad and an occlusive dressing. The occluded dressing was then removed after six hours and any residual test material was removed from the skin with a clean, dry, absorbent gauze pad.
Each animal was observed twice daily for clinical signs of toxicity and pharmacological effects. Body weights were recorded prior to dosing and then weekly throughout the study. The test site was examined daily and dermal reactions were graded using the Draize scale and recorded. At termination, blood samples were collected for the following clinical chemical and hematological determinations.

Hematology	**Clinical chemistry**
Erythrocyte count	Glucose
Total leukocyte count	Blood urea nitrogen
Differential leukocyte count	Total protein
Hemoglobin	SGOT
Hematocrit	SGPT

All animals were sacrificed and necropsied whether they had died or had survived throughout the study. Organs from animals found dead were not weighed but for animals surviving to the end of the study, the following organs were weighed and organ/body weight ratios were determined.
Heart, liver, spleen, kidneys, adrenals, thyroid, pituitary, testes, ovaries and brain.
The following tissues were collected and preserved and were prepared for subsequent histological examination.
Heart, Sacculus rotundus, Urinary bladder, lungs, colon, adipose tissue, bronchi, thymus, mammary gland, trachea, spleen, brain (cerebellum, cerebrum, pons), thyroid, liver, parathyroids, pancreas, pituitary, cervical lymph nodes, kidneys, spinal cord (two sections), salivary gland, adrenals, skeletal muscle, tongue, vagina, sciatic nerve, esophagus, seminal vesicles, testes/ovaries, skin (treated and untreated), stomach, bone, duodenum, epididymis, bone marrow (smear), jejunum, ileum, prostate/uterus, eyes, mesenteric lymph nodes and any gross lesions.

A two-tailed student's t-test was used to determine the significance of any differences between treated and control groups for body weights, clinical pathology and absolute and relative organ weights.

Result **:** One control male was found in a moribund state and was sacrificed on day 21 of the study. One female control was found dead on day 11 of the study.
One 1000 mg/kg/day male was found dead on day 15.
A male and a female in the highest dose group were found dead on days 10 and 24 respectively and the authors considered these to be treatment-related.
Clinical signs observed in the study that were considered to be treatment-related included: thinness, nasal discharge, lethargy, soiled anal area, anal discharge, wheezing.
There were group mean body weight losses in the mid and high dose groups and a smaller increase than controls in the low dose group as follows:

Group	Weight gain (kg) Males	Females
Control	0.3	0.3
200 mg/kg/day	0.2	0.2
1000 mg kg/day	0*	0*
2000 mg/kg/day	-0.3*	-0.4*

* P < 0.05

The authors judged that the weight losses in the mid dose group were not a direct effect of the test material. They noted that weight losses occurred in the first week of dosing and that after this there were increases in weight. The authors comment that such effects generally occur as a result of the stress of dosing and the dermal irritation that occurred.
The skin irritation grades showed that irritation was dose related and was greatest in the highest dose group. The mean irritation score, calculated as the mean sum of all the irritation scores (erythema and edema), for each group was as follows:

Group/sex	MIS	Classification
Control Male	0	Non-irritant
Control Female	0	Non-irritant
200 mg/kg/day Male	1.3	Slight irritant
200 mg/kg/day Female	1.7	Slight irritant
1000 mg/kg/day Male	3.7	Moderate irritant
1000 mg/kg/day Male	3.5	Moderate irritant
2000 mg/kg/day Male	4.1	Moderate irritant
2000 mg/kg/day Male	3.6	Moderate irritant

Other dermal findings included cracked, flaky and/or leathery skin, crusts and/or hair loss. These findings only occurred in the treated groups and appeared with greater frequency as the dose level increased.

There were no hematological findings in the female groups.
In males, reductions in RBC, hemoglobin and hematocrit were as shown. The actual mean values are shown for the controls and the % reductions are shown for the treated groups. Those values indicated * are significantly different (P<0.05).

	Control	200 mg/kg	1000 mg/kg	2000 mg/kg
RBC	7.1	-13%*	-20%*	-11%
Hemoglobin	14.9	-6%	-15%*	-15%*
Hematocrit	45.2	-7.7%	-21%*	-16%*

No treatment-related changes were seen in the clinical chemistry data with the following exceptions
Total protein reduced by 8% in 200 mg/kg males; SGPT and ALP reduced by 37% and 46% respectively in the 2000 mg/kg females. The authors comment that these values were well within the normal range of historical controls and are not judged to be test material related.

Organ weights and organ/body weight ratios differed from controls as shown in the following table. Values are shown as % differences, either greater (+%) or less (-%) than corresponding controls. Note that all values have been rounded to nearest whole number and only those values significantly different from controls are shown. No differences were observed for any other organ weight.

Organ	Dose mg/kg	Absolute organ wt M	Absolute organ wt F	Relative organ wt M	Relative organ wt F
Heart	200	+17%		+18%	
	1000	+22%		+32%	+35%
	2000				+35%
Liver	200				
	1000				
	2000	-24%			
Spleen	200		+128%		+125%
	1000	+83%	+91%	+75%	+125%
	2000			+50%	
R Kidney	200				
	1000				+20%
	2000			+13%	+28%
L Kidney	200				
	1000				+15%
	2000			+60%	+19%
R Adrenal	200		+55%		+50%
	1000				
	2000		+67%		+100%
L Adrenal	200				+75%
	1000				
	2000		+54%		+100%
Pituitary	200				
	1000				
	2000		+21%		+100%
Thyroid	200				
	1000				
	2000				
Brain	200				
	1000				
	2000			+21%	+21%
R Testis	200				
	1000				
	2000				
L Testis	200				
	1000				
	2000				
R Ovary	200				
	1000				
	2000				
L Ovary	200				
	1000				
	2000				

The authors concluded that the increases in relative heart weights for the mid- and high- dose males and females were treatment-related. Other heart weight changes were within the normal range for control values for the laboratory . Increased absolute and relative spleen weights for males were considered incidental since they fell within the normal range for the laboratory. For the females however, the differences were considered to be treatment-related. In both males and females, differences in absolute and relative adrenal weights were considered to be stress-related and therefore, indirectly related to treatment.

Gross necropsy findings were confined largely to the skin. Enlarged spleens in the female groups were also noted.

Microscopic pathology
Slight to moderate proliferative and slight to moderately severe inflammatory changes were present in the treated skin of all male and female animals in the high dose group. These changes were accompanied by an increase in granulopoiesis of the bone marrow in 5/6 males and 3/4 females.
4/6 high dose group males also had multifocal or diffuse tubular hypoplasia of a few of the seminiferous tubules of both testes. The degree of spermatogenesis was similar to controls in one animal, was absent in two animals and was slightly reduced in three animals. These testicular changes were considered by the authors to be secondary to the skin and/or weight changes.
All other lesions observed were considered to be incidental and unrelated to treatment.

Reliability : (1) valid without restriction
This study was audited by a quality assurance unit and was found to be satisfactorily conducted and reported.

(15)

Type	:	Sub-chronic
Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	Six hours each day
Frequency of treatm.	:	Daily, five days per week for 13 weeks
Doses	:	165, 330 & 495 mg/kg/day
Control group	:	Yes, concurrent vehicle
Year	:	1997
GLP	:	Yes
Test substance	:	Hydrodesulfurized kerosene

Method : Groups of 12 male and 12 female, individually housed, Sprague-Dawley rats aged 7-9 weeks were used. The males weighed 198-328 g and the females weighed 156-249 g at the initiation of the study.
Test material was applied at concentrations of 20, 40 or 60% (v/v) at a rate of 1 ml/kg/day to the shorn intrascapular region of the rats. This was equivalent to doses of test material of 165, 330 or 495 mg/kg/day. Dosing was continued daily for five consecutive days each week, five days a week for 13 weeks. In addition a group of 12 male and 12 female rats of similar age were administered mineral oil at a dose rate of 1 ml/kg/day; these animals served as vehicle controls. An additional 12 rats/sex/group in the vehicle controls and high dose group were maintained for a 4-week recovery period following dosing for 13 weeks. All animals were fitted with collars to prevent ingestion and these were removed six hours after dosing and any residual test or control material was wiped from the skin. Animals were observed for clinical signs prior to dosing and 1, 6 and 24 hours after the first dose. Subsequently, observations were made prior to each dose being applied.
Prior to the administration of each dose, the treated skin site was evaluated for dermal irritation using the Draize scoring method. Body weights were recorded prior to the first dose and weekly thereafter.
An ophthalmic examination was conducted on each rat prior to application of the first dose and again prior to sacrifice at the end of the study.

During the week prior to the first dose, each rat was subjected to a functional observation battery (FOB). The FOB was conducted again 1, 6 and 24 hours after the first dose and at 7 and 14 days. During the study, the FOB, motor activity and startle response testing was conducted on all rats at weeks 4, 8 and 12.
[The details of the FOB, the test for startle response test and the test for motor activity are given in detail in the laboratory report but are not included here].

At the 14 week necropsy, blood samples were collected from 12 animals/sex/group and at the week 18 necropsy from the recovery rats (vehicle and high dose groups).
The following hematological and clinical chemical parameters were measured.

Hematology
Erythrocyte count
Hemoglobin
Hematocrit
Mean corpuscular volume
Mean corpuscular hemoglobin
Mean corpuscular hemoglobin concentration
Platelet count
Reticulocyte count
Total leukocyte count
Differential leukocyte count
Morphological examination of erythrocytes and platelets
Coagulation determinations (prothrombin time & activated partial thromboplastin time) were also carried out on six animals from each group at week 14 and from the recovery groups at the week 18 necropsy.

Clinical chemistry
Blood urea nitrogen
Creatinine
Serum aspartate aminotransferase
Serum alanine aminotransferase
Alkaline phosphatase
Lactate dehydrogenase
Sorbitol dehydrogenase
Gamma glutamyl transferase
Creatinine kinase
Serum glucose
Total, direct and indirect bilirubin
Total protein
Albumin
Calcium
Phosphorus
Sodium
Potassium
Chloride

A complete necropsy was performed on six rats/sex/group following 13 weeks dosing, and on 6 rats/sex/group of the recovery animals (high dose and controls) at week 18. A limited necropsy was performed on the remaining six animals and their organs were not weighed (see below). Each full necropsy included an examination of the external surface of the body, all orifices, cranial, thoracic, abdominal and pelvic cavities and their contents. Gross observations were recorded and the following organs

were weighed:
Adrenal glands, brain, epididymides, heart, kidneys, liver, ovaries, prostate, spleen, testes, thymus and uterus.

The following tissues were collected, processed and then examined microscopically.

Adrenal glands	Nose (nasal cavity & turbinates)
Animal identification	Ovaries
Bone marrow (from sternum)	Oviducts
Brain	Pancreas
Epididymides	Parathyroid glands
Esophagus	Pituitary gland
Exorbital lacrimal glands	Prostate
Eyes with optic nerve	Salivary glands
Femur (incl. articular surface)	Seminal vesicles
Gross lesions	Skin (application site)
Harderian gland	Skin (inguinal)
Heart and aorta	Spinal cord (3 levels)
Intestine (3 levels)	Spleen
Kidneys	Stomach
Larynx and pharynx	Testes
Liver	Thymus
Lungs with mainstream bronchi	Thyroid gland
Lymph nodes (mandibular/mesenteric)	Urinary bladder
Mammary glands with adjacent skin	Uterus
Muscle (thigh)	Vagina
Nerve (sciatic)	

The remaining six rats of each group were anesthetized with an intraperitoneal injection of Pentothal ® and transcardially perfused in-situ using 10% neutral-buffered formalin and given a limited necropsy. For these rats, no organs were weighed and the following tissues were collected:

Head/skull	Sural nerve
Brain	Tibial nerve
Spinal cord	Gross lesions
Sciatic nerve	

The following tissues were examined microscopically in these animals:
Brain (forebrain, cerebrum, midbrain, cerebellum, pons and medulla obligata)
Gasserian ganglia
Dorsal root ganglia
Dorsal and ventral root fibers
Sural nerve
Tibial nerve
Spinal cord (cervical and lumbar areas)
Sciatic nerve.

Statistics
Normally-distributed in-life data (parametic) were analyzed for test substance effects by analysis of variance and pairwise comparisons made between groups using Dunnett's test. Nonparametric data (nonhomogenous as determined by Bartlett's) were analyzed using a modified t-test. Statistical significance was reported at the $P < 0.05$ level. Statistical analyses of neurobehavior data (FOB and motor activity) are described in the results section.

Result : All animals survived until scheduled termination.

There were no test substance-related effects on survival, clinical observations (apart from skin irritation), neurobehavioral signs or ophthalmological findings. The only clinical observations during the study were related to skin irritation at the application site. There was a generally dose-related increase in the incidence and severity of erythema, edema, epidermal scaling, scab formation, thickening of the skin and ulceration at the treated site. Males seemed to be more sensitive than females.
The FOB screen did not demonstrate any substance-related effects. The areas monitored were: behavioral parameters, including autonomic, muscle tone and equilibrium, sensorimotor responses, central nervous system. In addition the test substance had little effect on motor activity or startle response.
Growth rates were unaffected by treatment.
At necropsy no substance-related observations were made for males in any group. In the females there was a suggestion of a possible treatment-related effect which occurred in 7 rats across all groups and consisted of skin crusts or ulceration at the site of application of test material.
Hematological and serum clinical parameters were unaffected by treatment.
The only organ weight effects noted were an increase in spleen/body weight and spleen/brain weight ratios in the high dose group females at the 13 week necropsy and an increase in absolute spleen weight in the same dose group females after the 4 weeks recovery period. Since there were no associated microscopic or clinical chemical findings, these differences were not considered to be of biological relevance.
There were no treatment-related microscopic changes in the tissues examined with the exception of the findings in the skin. The skin observations were minimal in nature with a severity score less than 1 on a 1 [low] to 4 [severe] scale. The findings included acanthosis, ulceration, parakeratosis, chronic active inflammation and hyperkeratosis. The males were affected at all doses, however, the effects indicated very little irritation. Recovery group animals revealed complete recovery in the females and minimal hyperkeratosis in the high dose group males.
No effects were found in the animals subjected to a detailed neuropathological examination.

Test substance : The Hydrodesulfurized kerosene had the following properties.

Boiling point	148.9 °C (300 °F)
Specific gravity	0.825 @ 60 °F
Melting point	Not applicable
% volatile	100
Vapor pressure	0.4 mm Hg @ 68 °F
Evaporation rate (water = 1)	Slower
Vapor density (air = 1)	4.7
Viscosity	1.3 - 2.2 cSt @ 100 °F
% solubility in water	Negligible
Pour point	-34.4 °C (-30 °F)
pH	Not determined
Appearance/odor	Clear liquid with hydrocarbon odor

The vehicle used was Squibb mineral oil.

For dosing, mixtures of hydrodesulfurized kerosene were prepared in the mineral oil at concentrations of 20, 40 and 60% (v/v)

Reliability : (1) valid without restriction

(31)

Route of admin. : Dermal

Result : Five additional dermal repeat dose studies have been reported for materials in this group.
The results are summarized below. It should be noted that the 13 week study was used only as a dose range finding study and did not provide information on systemic toxicity, since it was designed to assess the suitability of the dosing regime with respect to skin irritation.

Study 1

Test material:	Hydrodesulfurized kerosene
Species:	Rabbit
Duration:	28 days
Dosing regime:	3x/week
Doses:	200, 1000 and 2000 mg/kg/day
Findings:	Skin irritation only. Other findings were either incidental or secondary to skin irritation or reduced body weight gain.
Reference:	API 30-32297
Reliability:	1

Study 2

Test material:	Jet Fuel A
Species:	Rabbit
Duration:	14 days
Dosing regime:	5x/week
Doses:	6.4 g/kg/day
Findings:	Severe skin damage at the treatment areas. Depression and weight loss associated with anorexia. Tissue damage in liver (mottled necrosis and centrilobular degeneration), kidney and bladder (hyperplasia) considered to be secondary to severe skin irritation
Reference:	API 27-32815
Reliability:	1

Study 3

Test material:	Hydrotreated straight run kerosene
Species:	Mouse
Duration:	3 weeks
Dosing regime:	3x/week
Doses:	Dose not specified
Findings:	Degenerative skin changes including necrosis and hyperplasia. The effects were well advanced after one week.
Reference:	Data summarized in CONCAWE 91/51
Reliability:	4, Reported as a review and without sufficient experimental detail.

Study 4

Test material:	3 kerosene samples: two hydrotreated, straight run kerosenes and a blend of 70% hydrocracked kerosene and 30% hydrotreated straight run kerosene
Species:	Mouse
Duration:	1 weeks

Dosing regime:	3 applications in one week
Doses:	Dose not specified
Findings:	Minimal macroscopic changes. Earliest changes were inflammation and necrosis of the hair follicles with subsequent degeneration at the skin surface
Reference:	Data summarized in CONCAWE 91/51
Reliability:	4, Reported as a review and without sufficient experimental detail.

Study 5

Test material:	2 kerosene samples: a hydrotreated, straight run kerosenes and a blend of 70% hydrocracked kerosene and 30% hydrotreated straight run kerosene
Species:	Mouse
Duration:	13 weeks
Dosing regime:	Various regimes. Purpose was to determine a dosing regime that would be suitable for a long-term study.
Doses:	100 or 50 µl/application
Findings:	Samples were highly irritating when 50 µl applied undiluted twice/week. Slightly less irritation occurred when 100 µl applied once/week. A 25% solution in white oil applied at a dose of 50 µl, twice/week was non-irritant. At necropsy no treatment-related effects were observed except those occurring in the skin.
Reference:	Data summarized in CONCAWE 93/55
Reliability:	4, Reported as a review and without sufficient experimental detail.

(8) (11) (37) (38)

Type	:	Sub-chronic
Species	:	Rat
Sex	:	Male/female
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Exposure period	:	Four weeks
Frequency of treatm.	:	Six hours/day, five days/week for four consecutive weeks
Doses	:	Nominal: 32 mg/m³. Actual: 24 mg/m³
Control group	:	Yes
Year	:	1986
GLP	:	yes
Test substance	:	API 81-09 (Hydrodesulfurized kerosine)

Method : Groups of 20 male and 20 female Sprague Dawley rats (approximately six weeks old) were exposed to a nominal concentration of 25mg/m³ kerosene by inhalation. Exposures were for approximately six hours each day, five days each week for four consecutive weeks. Control groups of 20 male and 20 females were exposed to filtered air.
Animals were observed twice daily for overt signs of toxicity and they underwent a detailed examination once weekly. Body weights were also recorded weekly. At study termination, the animals were killed and blood samples were taken for the following clinical chemical and hematological investigations:

Hematology	**Clinical chemistry**
Hematocrit	Aspartatae aminotransferase
Hemoglobin	Alanine aminotransferase
Erythrocyte count	Alkaline phosphatase
MCH	Glucose
MCV	Urea nitrogen
MCHC	Total protein
Leukocyte count	
Platelet count	
Reticulocyte count	

For all rats, the following organs were weighed and the organ body weight ratios were calculated :
Heart, lung and trachea, liver, kidneys, brain, spleen, adrenals, thyroid/parathyroid, pituitary, testes and ovaries.
The following tissues were removed and preserved:

Adrenals (2)	Aorta
Bone marrow (femur)	Bone marrow smear
Brain (3 levels)	Eye with contiguous Harderian gland
Esophagus	Stomach
Duodenum	Jejunum
Ileum	Cecum
Colon	Rectum
Gonads	Ovary (2)
Kidney (2)	Testis with epidiymis (2)
Heart	Liver (3 sections)
Nasal tissues	Lung & trachea (all lobes)
Abdominal lymph nodes	Thoracic lymph nodes
Mammary region lymph nodes	
Pancreas	Pituitary
Sciatic nerve	Prostate & seminal vesicle
Skeletal muscle (thigh)	Skin
Salivary gland (mandibular with submandibular lymph node)	
Spinal cord (cervical, midthoracic & lumbar)	
Spleen	Thymic region
Thyroid/parathyroid complex	
Urinary bladder	Uterus (2 horns & cervix)
Vagina	

The following tissues were examined microscopically in all rats:
Adrenal (2), brain (3 levels: fore, mid & hind), bronchi, esophagus, eye (2), heart, kidney (2), liver, lungs (2), lymph node (mediastinal), ovary (2), pancreas, pituitary, prostate, salivary gland, skin, spleen, stomach, testis (2), thymus, thyroid/parathyroid, trachea, urinary bladder, uterus, all gross lesions.

Statistical analysis
Body weight, hematology, clinical chemistry and organ weight data were analyzed by analysis of variance and Bartlett's test.
Treatment groups were compared to control by sex, using the appropriate t-statistic.
Data containing inequalities or where group variances were heterogenous were compared using a non parametric approach, by transforming the data into ranks prior to analysis as described by Conover and Iman.

Result **:** There were no treatment-related effects on clinical condition, growth rate organ or organ body weight ratios or on any of the hematological or clinical chemistry determinations.

Furthermore, there were no treatment-related microscopic changes observed in any of the organs examined.

Test condition : Atmospheres were generated by atomizing the test material into an atomization chamber. The resulting vapors/aerosols were directed to the chamber inlet where dilution with chamber ventilation air reduced the concentration to the desired level.
Nominal concentrations were calculated from test material use rates.
Actual concentrations were determined at approximately hourly intervals using a Total Hydrocarbon analyzer.
The nominal and actual concentrations for the study were:

	Exposure concentration (mg/m³)			
Desired concentration (mg/m³)	Nominal Mean	SD	Actual Mean	SD
25	32	1.74	24	1.61

Reliability : (1) valid without restriction

(20)

5.5 GENETIC TOXICITY 'IN VITRO'

Type : In vitro studies

Remark : The In vitro genotoxic potential of kerosenes and jet fuel has been studied in several studies and these are tabulated below. Only one study for each test system is described in detail in the following section on in vitro genotoxicity studies and these are indicated (*) in the table.
For completeness, the results of all studies are briefly summarized as separate robust summaries. At the end of this section the results of all in vitro studies are summarized in tabular form.

Study type	Test material	Reference
Ames assay		
	Deodorized kerosene	API 26-60103
	Straight run kerosene	API 26-60017*
	Jet Fuel A	API 27-30051
Modifed Ames assay		
	Straight run kerosene	CONCAWE 91/51*
	Hydrotreated kerosene (3 samples)	CONCAWE 91/51
	Straight run kerosene	Blackburn et al '86
	Hydrotreated kerosene	Blackburn et al '86
Mouse lymphoma assay		
	Straight run kerosene	API 32-32745
	Straight run kerosene	API 26-60017
	Hydrodesulfurized kerosene	API 32-30240*
	Jet Fuel A	API 27-30051
	Jet Fuel JP-5	NTP 1986
Sister chromatid exchange assay (CHO cells)		
	Hydrodesulfurized kerosene	API 35-32482*

Type : Ames test
System of testing : S. typhimurium TA1535, TA1537, TA1538, TA98 & TA100 and S. cerevisiae D4
Test concentration : 1/8, 1/4, 1/2 & 1/1 of LD50 equivalent to 0.001 - 5.0 µl/plate
Cycotoxic concentr. : LD50 = 5% in DMSO
Metabolic activation : With and without
Result : Negative
Year : 1977
GLP : No data
Test substance : Kerosene

Method : The test solvent used was DMSO.
The standard Ames plate and suspension assays were conducted with and without metabolic activation. Araclor induced rat liver microsomes were used for metabolic activation.
Cytotoxicity of the test material was determined prior to the assay i.e the LD_{50} was determined and was found to be 5%.
The concentrations used in the mutagenicity assays are shown below. The lowest concentration used was below a concentration that demonstrated any toxic effects.

Test dose	**Percent concentration**	
	Bacteria	**Yeast**
1/8 50% survival	0.625	0.625
1/4 50% survival	1.25	1.25
1/2 50% survival	2.5	2.5
50% survival	5.0	5.0

The following positive control materials were used:
Non-activation assay

Substance	Solvent
Ethylmethane sulfonate	Water or saline
Methylnitrosoguanidine	Water or saline
2-Nitrofluorene	DMSO
Quinacrine mustard	Water or saline

Activation assay

2-Anthramine	DMSO
2-Acetylaminofluorene	DMSO
8-Aminoquinoline	DMSO
Dimethylnitrosamine	Water or saline

Result : All results from the plate assays and the suspension assays were negative both in the presence and absence of metabolic activation.
Test substance : The sample of kerosene was characterized as follows:

API gravity	41.7°
Initial boiling point	350 °F
Final boiling point	519 °F
Reid vapor pressure	1.7 lb
Sulfur	111 ppm
Vapor density	6.5
Paraffins	32% (v/v)
Olefins	1% (v/v)
Naphthenes	49% (v/v)
Aromatics	18% (v/v)
C6 aromatics	-
C7 aromatics	-
C8 aromatics	-
C9+aromatics	18% (v/v)

Reliability : (3) invalid
The standard Ames assay is an unsuitable test system for insoluble petroleum products.

(3)

Type : Ames test
System of testing : S. typhimurium TA98 & TA100
Test concentration : 10 µl/plate
Cycotoxic concentr. : Not stated
Metabolic activation : With
Result : Negative
Year : 1978
GLP : No data
Test substance : Deodorized kerosene

Reliability : (3) invalid
Report contains few details.
Furthermore, this assay has been shown to be an inappropriate method for insoluble petroleum distillates.

(4)

Type : Ames test
System of testing : S. typhimurium TA 1535, TA1537, TA1538, TA98 & TA100
Test concentration : 245 to 41 000 µg/plate
Cycotoxic concentr. : > 40 000 µg/plate in ethyl acetate
Metabolic activation : With and without
Result : Negative
Year : 1979
GLP : No data
Test substance : Jet fuel A

Reliability : (3) invalid
This assay has been shown to be an inappropriate method for insoluble petroleum distillates.

(5)

Type : Modified Ames assay
System of testing : S. typhimurium TA98
Test concentration : 50 µl/plate
Cycotoxic concentr. : No data
Metabolic activation : With
Result : Negative
Year : 1991
GLP : No data
Test substance : Kerosene, 3 samples

Method : The method described by Blackburn et al (1986) was used to determine the mutagenicity index of three samples of kerosene. This method differs from the standard Ames assay in the following respects:

- A DMSO extract of the test material is used
- The S9 fraction is obtained from Araclor-induced hamster liver
- An eight-fold concentration of S9 is used
- A two-fold concentration of cofactor NADP is used
- The assay is only conducted in S. typhimurium strain TA98

The DMSO extracts were tested over a range of concentrations that permitted the construction of a dose-response curve.

A Mutagenicity index (MI) was determined for each assay. This was the tangent to the dose-response curve at zero dose.
An assay was judged to be positive if the MI was greater than 1.0

Result : The mutagenicity indices of the three samples were all zero, thus demonstrating an absence of mutagenic activity. Interestingly, none of the samples was found to contain any measurable 3-7 ring PACs.

Test substance : Three samples were tested. All were straight run kerosenes:

Sample	CAS No.	Treatment	Crude source
1	8008-20-6	wet treated	Middle East
2	64742-81-0	hydrotreated	Middle East
3	64742-81-0	hydrotreated	North Sea

Reliability : (4) not assignable
The results are given as a summary in a review. No experimental details are provided.

(32) (37)

Type : Modified Ames assay
System of testing : S. typhimurium TA98
Test concentration : 50 µl/plate
Cycotoxic concentr. : No data
Metabolic activation : With
Year : 1986
GLP : No data
Test substance : 2 Samples: Hydrotreated kerosene & Straight run kerosene

Result : The mutagenicity index reported for each of the samples was

Straight run kerosene (8008-20-6) MI 2.9
Hydrotreated kerosene (64742-47-8) MI 0.0

Reliability : (4) not assignable
Paper is a compilation of results of many petroleum distillates. No actual data are provided.

(32)

Type : Mouse lymphoma assay
System of testing : Mouse lymphoma L5178Y cell line
Metabolic activation : With and without
Result : Negative
Year : 1984
GLP : Yes
Test substance : Hydrodesulfurized kerosene Sample API 81-07

Method : Non-Activation assay
Cultures of mouse lymphoma cells were exposed to the test material for four hours at doses that were selected during a cytotoxicity study that had been carried out previously. Following exposure, the cells were washed and placed in growth medium for two or three days to allow recovery, growth and expression of the induced TK-/- phenotype. Cell counts were made daily and appropriate dilutions were made to allow optimal growth rates.
At the end of the expression period, 3×10^6 cells for each dose were seeded in soft agar plates with selection medium and resistant (mutant) colonies were counted after 10 days incubation. To determine the actual number of cells capable of forming colonies, a portion of the cell

suspension was also cloned in normal (non-selective) medium. The ratio of resistant colonies to total viable cell number is the mutant frequency.

Activation Assay
The activation assay was run concurrently with the non-activation assay. The only difference was the addition of the S9 fraction of rat liver homogenate and necessary co factors during the four hour treatment period.
The final concentrations of the activation system components in the cell suspension were:
2.4 mg NADP/ml; 4.5 mg isocitric acid/ml; 50 µl S9/ml.

S9 homogenate was obtained from Araclor-induced rat liver.

Evaluation criteria
The minimum condition considered necessary to demonstrate mutagenesis for any given treatment is a mutant frequency that exceeds 150% of the concurrent background frequency by at least 10 x 10^{-6}

Result **:** The test material was immiscible with water and DMSO at 100 µl/ml but was miscible with ethanol at the same concentration.
Stocks were prepared by performing serial dilutions in ethanol just prior to each assay. The mutation assays were then initiated by performing final dilutions of the stocks into the assay medium containing the lymphoma cells. The test material appeared soluble in the assay medium up to 125 nl/ml but a white precipitate was noted from 250 to 1000 nl/ml.

Two trials of the assay were initiated.
Trial 1 was performed with and without activation but the non-activation assay was NOT used in the evaluation because of unnacceptable suspension growth in the negative controls.
The non-activation portion of the assay was therefore repeated in Trial 2.

The report summarized here included the acceptable activation assay from Trial 1 and the acceptable non-activation assay from Trial 2.

The results are summarized below.

	Rel Susp. growth (% of control)	**Total mutant colonies**	**Total viable**	**Cloning eff.**	**Rel growth (%)**	**Mutant frequency $10E^{-6}$ units**
Non activation assay (Trial 2)						
Solvent control (ethanol)						
	100	70	392	100	100	17.9
	100	74	287	100	100	25.8
	100	75	367	100	100	20.4
	100	50	324	100	100	15.4
Untreated control						
	107.1	82	374	109.2	116.7	21.9
	97.4	70	392	116.5	111.3	17.9
EMS (µl/ml)						
0.5	46.7	851	170	49.6	23.2	500.6
0.5	57.3	753	155	45.3	25.9	485.8
API 81-07 (nl/ml)						
6.25	28.3	83	331	96.6	27.3	25.1
12.5	24.7	61	282	82.3	20.3	21.6

12.5	67.4	89	332	96.9	65.3	26.8
25.0	16.3	87	258	75.3	12.3	33.7
25.0	45.4	61	248	72.4	82.7	24.6
37.5	10.3	105	366	106.8	11	28.7
37.5	6.2	77	143	41.7	2.6	53.8
Activation assay						
Solvent control (ethanol)						
	100	98	236	100	100	41.5
	100	92	282	100	100	32.6
Untreated control						
	128.2	99	226	87.2	111.8	43.8
DMN (µl/ml)						
0.3	58.5	151	34	13.1	7.7	444.1
API 81-07 (nl/ml)						
3.91	100.1	66	194	74.8	74.9	34
7.81	194.1	52	144	55.6	107.9	36.1
15.6	101.7	42	259	99.9	101.6	16.2
31.3	27.3	45	158	61	16.7	28.5
62.5	9	68	175	67.5	6.1	38.9

Under non-activation conditions the test material induced a good range of toxicities for evaluation (relative growths ranged from 2.8% to 65.3%). None of the assays induced a mutant frequency that exceeded the minimum criterion (40.8×10^{-6}). The test material was not mutagenic under non-activation conditions.
In the presence of metabolic activation a wide range of toxicities was induced (6.1 to 107.9% relative growths). The minimum criterion mutant frequency of 69.0×10^{-6} was not exceeded. The test material was therefore considered non mutagenic under activation conditions.

Reliability : (1) valid without restriction

(14)

Type : Mouse lymphoma assay
System of testing : Mouse lymphoma cells L5178Y cell line
Test concentration : 0.5 to 00067 µl/ml
Cycotoxic concentr. : 100% mortality at 0.075 µl/ml, 10% mortality at 0.01 µl/ml
Metabolic activation : With and without
Result : Positive
Year : 1985
GLP : Yes
Test substance : Straight run kerosene, API sample 83-09

Reliability : (1) valid without restriction

(17)

Type : Mouse lymphoma assay
System of testing : Mouse lymphoma cells L5178Y cell line
Test concentration : With activation: 0.004 to 0.065 µl/ml, without activation: 0.006 to 0.13 µl/ml
Cycotoxic concentr. : Toxic at 0.13 µl/ml, slightly toxic at 0.065 µl/ml
Metabolic activation : With and without
Result : Negative
Year : 1977
GLP : No data
Test substance : Straight run kerosene
Reliability : (1) valid without restriction

(3)

Type	:	Mouse lymphoma assay
System of testing	:	Mouse lymphoma cells L5178Y cell line
Test concentration	:	With activation:25 to 200 µg ml, without activation 100 to 1200 µg/ml
Cycotoxic concentr.	:	Max concentration that could be used was 0.1%
Metabolic activation	:	With and without
Result	:	Positive
Year	:	1979
GLP	:	No data
Test substance	:	Jet fuel A

Result : Negative in the non-activation assay.
Positive in the activation assay.

Reliability : (1) valid without restriction

(5)

Type	:	Mouse lymphoma assay
System of testing	:	Mouse lymphoma cells L5178Y cell line
Test concentration	:	10 mg/plate
Result	:	negative
Year	:	1986
Test substance	:	Jet fuel JP-5

(61)

Type	:	Sister chromatid exchange assay
System of testing	:	Chinese Hamster Ovary cells
Metabolic activation	:	With and without
Result	:	Negative
Year	:	1988
GLP	:	Yes
Test substance	:	Hydrodesulfurized kerosene, API sample 81-07

Method : A cytotoxicity study was performed in order to select dose levels for the SCE assay.
For the SCE assay CHO cells were seeded in duplicate for each treatment condition and were incubated at 37°C in a humidified atmosphere for 16 to 24 hours.
Treatment was carried out by refeeding two complete sets of flasks with complete medium for the non activation study or with S-9 reaction mixture for the activated study to which was added 50 µl of dosing solution of test control or article in solvent or solvent alone.
In the non activation study the cells were exposed for about 25 hours. At the end of the treatment period, the treatment medium was removed, the cells rinsed and then exposed to 0.01mM BrdUrd and colcemid (0.1 µg/ml) for a further 2 hours.
In the activation study exposure was for 2 hours.
After the exposure period, the treatment medium was removed, the cells were washed re-fed with medium containing BrdUrd and then incubated for a further 26 hours. Colcemid was added for the last 2 hours of incubation.
For activated and non activated assays metaphase cells were harvested 2 hours after addition of colcemid. Cells were collected and fixed and stored until slides were prepared.
Slides were coded and scored without regard to treatment group. Only cells with 20 ±2 centromeres were selected for evaluation of SCEs. A total of 4 doses were scored including the highest test article dose where sufficient second-division metaphase cells wee available. SCEs were

scored in 25 cells from each duplicate culture to make up a total of 50 cells per treatment. The percentage of cells in first (M1), second (M2) or third division (M3) metaphase was also recorded for a total of 100 metaphase cells scored.
TEM was used as positive control in the non activated assay at a concentration of 0.025 µg/ml. CP was used in the activation assay at a concentration of 2.5 µg/ml.

S-9 was prepared from Araclor-induced rat liver.

The test material was considered positive if it induced a doubling in SCE frequency over the solvent control at a minimum of three consecutive dose levels or if a dose responsive and statistically significant increase was observed over a minimum of three dose levels.

Result **:** The test material was soluble at all concentrations tested.
The study in both the presence and absence of S9 was repeated since there was a poor metaphase cell yield. Only the results of the second study with and without S9 are summarized in the following table.

Replicate flask	**SCEs/ chromosome**	**Flask mean SCEs/cell (±SD)**	**Group mean SCEs/cell (±SD)**
Assay in absence of exogenous activation			
Untreated cells			
A	0.42	8.4±3.16	
B	0.42	8.28±2.57	8.34±2.85
Acetone			
A	0.48	9.44±3	
B	0.45	8.96±2.35	9.20±2.68
API 81-07	0.007 µl/ml		
A	0.44	8.80±2.87	
B	0.43	8.68±2.54	8.74±2.69
API 81-07	0.013 µl/ml		
A	0.47	9.4±2.96	
B	0.42	8.32±2.58	8.86±2.80
API 81-07	0.025 µl/ml		
A	0.47	9.36±2.96	
B	0.48	9.64±3.12	9.50±3.01
API 81-07	0.05 µl/ml		
A	NE [(a)]		
B	NE		
TEM			
A	1.53	30.6±6.81	
B	1.75	34.92±7.60	2.76±7.47**

Assay in presence of exogenous activation

Untreated cells			
A	0.5	10.16±2.98	
B	0.55	11.04±2.76	10.6±2.88
Acetone			
A	0.47	9.36±3.94	
B	0.45	8.96±2.99	9.16±3.47
API 81-07 0.05 µl/ml			
A	0.63	12.52±4.09	
B	0.58	11.64±3.38	12.08±3.74**
API 81-07 0.1 µl/ml			
A	0.48	9.56±3.8	
B	0.51	10.36±4.27	9.96±4.02
API 81-07 0.2 µl/ml			
A	0.5	10.04±3.23	
B	0.44	8.84±3.5	9.44±3.39
API 81-07 0.4 µl/ml			
A	0.5	9.96±3.25	
B	0.58	11.56±3.57	10.76±3.47*
CP			
A	1.91	38.2±7.06	
B	2.01	40.2±12.18	39.2±9.91**

(a) Not evaluated due to absence of second-division metaphase cells

* P</=0.05

** P</=0.01

The responses to the positive and negative control materials fulfilled the requirements for the assays.
The test material did not cause an increase in SCEs in the absence of exogenous activation.
API 81-07 did cause a significant increase in SCEs at two non adjacent doses in the activation assay. However, the increased activity was only seen in one of two treatment flasks. These increases appeared to be random and of no biological significance.
It was concluded that API 81-07 was negative in the SCE assay.

Reliability : (1) valid without restriction

(24)

Type : Overall summary of in-vitro studies
System of testing : Various

Result : Summarized results of all the reported in vitro studies of genotoxicity are shown below

Test material	Result +S9	Result -S9	Reference
Standard Ames assay			
Deodorized kerosene	-	-	API 26-60103
Straight run kerosene	-	-	API 26-60017*
Jet Fuel A	-	-	API 27-30051
Modified Ames assay			
Straight run kerosene (MD5)			
	-	ND	CONCAWE 91/51*
Straight run kerosene	+	ND	Blackburn et al 86*
Hydrotreated kerosene (MD 2, 3, & 4)			
	-	ND	CONCAWE 91/51
Hydrotreated kerosene	-	ND	Blackburn et al '86
Mouse Lymphoma assay			
Straight run kerosene (83-09)			
	± [(a)]	+	API 32-32745
Straight run kerosene	-	-	API 26-60017
Hydrodesulfurized kerosene (81-07)			
	-	-	API 32-30240*
Jet Fuel A	+	-	API 27-30051
Jet Fuel JP-5	-	-	NTP 1986
Sister chromatid exchange assay (CHO cells)			
Hydrodesulfurized kerosene (81-07)			
	-	-	API 35-32482*

[(a)] Equivocal with activation
* Studies for which full robust summaries have been prepared

5.6 GENETIC TOXICITY 'IN VIVO'

Type : In vivo studies

Remark : The in vivo genotoxic potential of kerosenes and jet fuel has been studied in several studies and these are tabulated below. Only one study for each test system is described in detail in the following section on in vivo genotoxicity studies and these are indicated (*) in the table.
For completeness, the results of all studies are also briefly summarized as separate robust summaries. At the end of this section the results of all in vivo studies are summarized in tabular form.

Assay	Reference
Rat bone marrow cytogenetics	
Hydrodesulfurized kerosene (81-07)	API 32-30240*
Straight run kerosene (83-09)	API 32-31769
Straight run kerosene (8008-20-6)	API 26-60017
Straight run kerosene (8008-20-6)	API 26-60017
Jet fuel A	API 27-30051*
Sister chromatid exchange	
Hydrodesulfurized kerosene (81-07)	API 36-30043*
Dominant lethal assay	
Deodorized kerosene	API 26-60098
Jet fuel A	API 28-31345*

* Full robust summaries prepared for these studies

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Doses : 0.3, 1.0 & 3.0 g/kg
Result : Negative
Year : 1984
GLP : Yes
Test substance : Hydrodesulfurized kerosene Sample API 81-07

Method : A pilot study was carried out in 4 male and 4 female young adult Sprague Dawley rats. These animals were given a single intraperitoneal (i.p.) dose (3 g/kg) of API 81-07. During the following 48 hours observation, no animals died. The doses selected for the cytogenetics study were therefore 0.3, 1 and 3 g/kg.
Three groups of 15 male and 15 female rats were given a single i.p. dose of either 0.3, 1 or 3 g API 81-07/kg. At six, 24 and 48 hours after dosing 5 males and 5 females were killed at each dose level. An additional 15 males and 15 females were untreated and served as negative controls. These animals were otherwise treated the same as the test animals. A positive control group of 5 males and 5 females was administered 0.8 mg/kg Triethylenemelamine (TEM) as a single i.p. dose. These positive control animals were killed 24 hours after administration of the positive

control substance.
Three hours prior to being killed with CO_2, animals were injected i.p. with 4 mg/kg of colchicine. After the animal was killed, the adhering soft tissue and epiphyses of both tibiae were removed and the marrow was flushed from the bone and transferred to Hank's balanced salt solution. The marrow button was collected by centrifugation and was then re suspended in 0.075M KCl. The centrifugation was repeated and the pellet re suspended in fixative (methanol:acetic acid, 3:1). The fixative was changed once and left overnight. Cells in fixative were dropped onto glass slides which were then air dried and stained with Giemsa. Slides were coded and scored for chromosomal aberrations.
50 spreads were read for each animal where feasible.
A mitotic index based on at least 500 counted cells was also recorded. The index was calculated by scoring the number of cells in mitosis per 500 cells on each read slide.

Statistical evaluation
Performed by Student's t-tests on four parameters:
1. Number of structural aberrations per animal
2. Number of numerical aberrations per animal
3. % cells with one or more structural aberrations per animal
4. % cells with 2 or more structural aberrations per animal.

Data interpretation and evaluation
Gaps were not counted as significant aberrations.
Open breaks were considered as indicators of genetic damage as were configurations resulting from the repair of breaks. The latter included translocations, multiradials, rings, multicentrics, etc. Reunion figures such as these were weighed slightly higher than breaks since they usually resulted from more than one break.
Cells with more than one aberration were considered to indicate more genetic damage than those with evidence of single events.
Consistent variations from the euploid number were also considered in the evaluation of mutagenic potential.

The type of aberration, its frequency and its correlation to dose in a given time was considered in evaluating the test material as being positive or negative.

Result : The data are given in the report for males, females and as male and female pooled data.
When the results for males were compared with those for controls and the females were compared to controls, no statistically significant differences were found. The data summarized below, are the pooled data for males and females.

Time after dose (hrs)	**No of rats**	**Total No of cells**	**% cells with aberrations 1+**	**2+**	**Mitotic index**
Negative control					
6	6	300	0	0	5.6
24	10	500	0.4	0	4.4
48	10	500	0	0	4.3
Positive control (TEM, 0.8 mg/kg)					
24	7	253	19.4**	10.7*	1.0

API 81-07, 0.3 g/kg

6	8	292	0.7	0	4.2
24	9	450	0	0	4.5
48	10	500	0	0	4.9

API 81-07, 1.0 g/kg

6	8	400	0.8	0.2	5.2
24	9	450	0	0	4.4
48	9	407	1.0	0.2	7.0

API 81-07, 3.0 g/kg

6	8	365	0.3	0	4.0
24	10	475	0.6	0	3.5
48	8	400	0	0	6.0

* Significant if outlier excluded
**P < 0.01

The structural aberration frequency did not differ significantly from the negative control at any tested dose. The percentage of cells showing one or more structural aberrations or 2 or more structural aberrations were also similar to the negative controls. A concurrent positive control group induced significant increases in aberrations.

Reliability : (1) valid without restriction

(14)

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : 6, 24 and 48 hours
Doses : 0, 0.3, 1.0 & 3.0 g/kg
Result : Negative
Year : 1985
GLP : Yes
Test substance : Straight run kerosene, API sample 83-09

Reliability : (1) valid without restriction

(18)

Type : Cytogenetic assay
Species : Rat
Sex : Male/female
Strain : Sprague-Dawley
Route of admin. : i.p.
Exposure period : 6, 24 & 48 hours
Doses : Sample 1: 0.4, 0.13 & 0.04 ml/rat; Sample 2:0.18, 0.06, 0.02 ml/rat
Result : Negative
Year : 1977
GLP : Yes
Test substance : Straight run kerosene, 2 samples

Result : Neither of the samples cause aberrations in bone marrow cells.
Reliability : (1) valid without restriction

(3)

Type	:	Cytogenetic assay
Species	:	Rat
Sex	:	Male
Strain	:	Sprague-Dawley
Route of admin.	:	Inhalation
Exposure period	:	Six hours each day for either five or 20 days
Doses	:	400 ppm (5 exposures), 100 ppm (20 exposures)
Result	:	Positive
Year	:	1979
GLP	:	No data
Test substance	:	Jet Fuel A

Method :

Groups of 5 sexually-mature Sprague-Dawley rats were exposed by inhalation six hours each day to Jet Fuel at concentrations of either 100 ppm for 20 days or 400 ppm for 5 days. A group of five males were exposed to air only and these served as controls. All animals were examined prior to and at hourly intervals during exposure and after each daily exposure session for evidence of unusual appearance and/or behavior. Body weights were recorded prior to exposure and at weekly intervals thereafter. The day after the last exposure for each group, the animals were administered colchicine intraperitoneally (2 mg/kg) to inhibit mitosis of dividing cells.

Two to four hours after administration of colchicine, the animals were sacrificed using carbon dioxide. Immediately following sacrifice, bone marrow cells were collected, by aspiration, from both femurs of each animal. The aspirates were collected into Hank's balanced salt solution and this was then centrifuged. The supernatant was discarded and the pellet was re-suspended in 0.075M KCl. After standing at room temperature for 25 minutes, each tube was centrifuged, the supernatant was discarded and Carnoy's fixative (3:1 methanol:glacial acetic acid vol:vol) was added to the pellet. The tubes stood at room temperature for 20 minutes and were then centrifuged, the supernatant was discarded and the pellet was re-suspended in fresh fixative. Each tube was sealed and stored in a refrigerator overnight at 4°C. The following day the tubes were again centrifuged, the supernatant was removed and the pellet again re-suspended in fresh fixative. Three drops of the final cell suspension were dropped onto microscope slides which were air-dried and then stained with Giemsa for microscopic examination.

Fifty cells in metaphase were examined from each rat. The metaphases were observed for the presence of cytogenetic abnormalities, mitotic index (No. of cells undergoing mitosis per 100 cells counted) and modal number (number of chromosomes in each metaphase).

Chromosomal aberrations were classified into one of four groups:

Chromatid breaks
Chromosome breaks
Markers which involve dicentrics, exchanges, rings and other miscellaneous configurations.
Severely damaged cells.

<u>Statistical analysis</u>

Mean change in body weights, mean modal number and mean mitotic index were analyzed using Bartlett's test for equality of variance and the one way analysis of variance. If a significant result was obtained, group mean values were compared using the multiple comparison procedure of Games and Howell. If only the ANOVA were significant, Scheffe's multiple comparison procedure was used to compare group means.

Aberrant cells and categories of chromatid breaks, chromosome breaks, markers and severely damaged cells were statistically analyzed using either the Wilcoxon nonparametric comparison of group means or a chi-square analysis. Regression analyses were performed on all data when significant responses were observed in the treated groups.

Result : The control animals exhibited normal appearance and behavior throughout the study and gained approximately 96 g body weight over the 20 day period.
The animals exposed to 400 ppm jet fuel for 5 days exhibited the following signs: they were restless and agitated during the first hour after each exposure and 2 to 5 exhibited signs of irritation in the muzzle area. As the study progressed there was an increasing incidence of respiratory distress and irritation around the nasal area. No deaths occurred during the study. There was a mean body weight loss of approximately 25 g in this group. Although the animals exposed to 100 ppm jet fuel appeared normal for the first 3 days of the study, nasal discharge were observed in 2 to 5 animals during exposures and after exposure termination. Two to five animals also showed signs of respiratory distress during exposures on the 5th, 6th, 7th and 8th days (characterized by sneezing). No deaths occurred during the study. Mean weight gain for this group was approximately 70 g.

Cytogenetic analysis
Although it was intended to analyze 50 metaphases for each animal (250 per group of 5 animals), due to a technical procedure failure, only 200 metaphases were analyzed in the group exposed to 400 ppm jet fuel for 5 days. This departure from the protocol is not considered to have negated the results of the study.

The percent aberrant cells per group and the percent of the four categories of aberrations are given below.

Dose	Total Aberrations	Chromatid Breaks	Percentage Chromosome Breaks	Markers	Severely Damaged
Control	9.6	3.2	0	6	0
400	22	7.5	0	10.5	5
100	19.2	11.2	0	9.6	0

The differences in the above table between the control values and those of the treated groups for chromatid breaks and markers were not statistically significant. However, The total aberrations for both the treated groups were significantly greater than the controls.

The number of chromosomes (modal number) was scored in each metaphase where possible and these results are shown in the following table.

No. of metaphases observed	No. cells Modal No. Determined	Mean Modal No.	Range
Control group			
250	250	41.1±0.46	40.5-41.62
400 ppm for 5 days			
200	197	39.6±0.62	38.94-40.41
100 ppm for 20 days			
250	250	41.1±0.59	40.20-41.80

Statistical analyses showed that the difference between the control and

400 ppm group for mean modal number was significant.

The mitotic indices are shown in the following table

Group	Mean Mitotic Index	Range
0 ppm	3.0±2.55	1-7
400 ppm	3.25±2.63	1-7
100 ppm	5.2±3.42	1-8

There were no statistically significant differences in the mean mitotic indices.

It was concluded that Jet fuel A had caused an increase in the total number of aberrations and was, therefore, positive in this assay

Test condition : Atmosphere generation was accomplished by metering measured volumes (20 cc) of test material, using a Harvard infusion pump, into a 500 ml 3-neck vaporization flask heated to 650°F. The resultant vapor was swept by an accessory airflow into a water-cooled condensing column. The condensing column was attached to the center neck of a 3-neck mixing flask. The cooled, diluted vapor was swept by an accessory airflow into the exposure chamber inlet. Accessory airflows for both the vaporization and mixing flasks were regulated by flowmeters. Control of the vapor concentration was facilitated through adjustments of the Harvard infusion pump settings and accessory airflows.

The chamber concentrations were monitored continuously during each 6-hour exposure period.
The target and mean actual concentrations were as follows:

Target concentration (ppm)	Actual Mean daily concentration (ppm)
400	389 ± 24.4
100	102 ± 7.6

Reliability : (1) valid without restriction

(5)

Type : Sister chromatid exchange assay
Species : Mouse
Sex : Male/female
Strain : B6C3F1
Route of admin. : i.p.
Exposure period : 20-22 hours
Doses : 400, 2000 & 4000 mg/kg
Result : Positive
Year : 1988
GLP : Yes
Test substance : Hydrodesulfurized kerosene, sample API 81-07

Method : Six groups of five male and five female, 8-10 week old B6C3F1 mice were anesthetized with Metofane and an agar-coated BrdUrd pellet was implanted subcutaneously in the lower abdominal region.

Four hours after implantation of the pellet the mice were given test material as a single i.p dose at doses of either 400, 2000 or 4000 mg/kg in corn oil at a dose volume of 10 ml/kg (doses based a range finding study). A solvent control group was given corn oil at a dose of 10 ml/kg. Two positive control groups were used. One received cyclophosphamide i.p. at

a dose of 10 ml/kg and the other received API 81-15 (a carcinogenic heavy fuel oil component) at a dose level of 4 g/kg, also at an injection rate of 10 ml/kg. Two to four hours prior to sacrifice, colchicine was injected intraperitoneally at a dose of 1 mg/kg to arrest dividing cells at metaphase. 24-26 hours after pellet implantation all mice were sacrificed by CO_2. Both femurs of each animal were cut and the marrow was aspirated into a syringe containing cold Hank's balanced salt solution. The cells were collected by centrifugation, resuspended in 5 ml warm hypotonic solution and then incubated for approximately 10 minutes at 37 °C to swell the cells. The swollen cells were collected by centrifugation, resuspended in two consecutive changes of methanol/acetic acid (fixative) and then stored in a refrigerator prior to slide preparation.
Slides were prepared by resuspending the cells in fresh fixative, by centrifugation and then dropping 2 to 4 drops of fixed cells onto a wet slide. The slides were then air-dried. Two to 5 slides were prepared from each animal. The slides were stained with Giemsa for subsequent examination.

A minimum of 50 second-division metaphase spreads from each animal were examined and scored for SCEs and chromosome number. The mitotic index was recorded as the % of cells in mitosis based upon 500 counted cells. The % first, second and third-division metaphase cells was also recorded as the number per 100 counted cells.

Using the number of animals per group as the sample size, statistical analysis was performed with the Kruskall Wallis test and, if required, the Mann Whitney test.

The test article was considered to induce a positive response if a dose-related increase in SCEs/metaphase was observed relative to the vehicle control ($P<0.05$, Mann Whitney test).

Criteria for determination of a valid test
The mean number of SCE per second-division metaphase cell must not exceed 8 SCEs/cell in the negative (vehicle) control. The mean SCE/metaphase for the positive control animals must be statistically increased relative to the vehicle control using the Mann Whitney test ($P<0.05$).

Result : The high dose male mice were lethargic shortly after dose administration and on the following day. In the high dose males and females and the mid-dose males there was a slight weight loss between the time pretreatment body weights were measured and when the animals were treated with colchicine on the following day.
The body weight changes are shown below:

Treatment	**Sex**	**% change (+ or -) 24 hr**
Corn oil 10 ml/kg	M	0
	F	- 2.9
API 81-07	M	- 1.4
400 mg/kg	F	- 0.5
2000 mg/kg	M	- 3.5
	F	- 1.9
4000 mg/kg	M	- 4.3
	F	- 4.7

API 81-15	M	0
4000 mg/kg	F	+ 3.8
Cyclophosphamide		
10 mg/kg	M	+ 2.6
	F	- 3.8

The SCEs were counted in 50 second-division metaphase cells and these data are summarized in the following table.

Treatment	Sex	Range of mean SCEs/cell for individual animals	Average SCEs/cell per mouse [(a)]
Corn oil	M	4.68 - 5.84	5.44±0.47 (5.64)
	F	5.28 - 7.36	6.25±0.86 (6.06)
API 81-07			
400 mg/kg	M	6.76 - 10.46	8.26±1.51 (5.64)**
	F	6.98 - 8.26	7.56±0.52 (7.56)
2000 mg/kg	M	5.68 - 7.58	6.74±0.87 (6.86)**
	F	6.8 - 9.84	8.26±1.22 (8.54)
4000 mg/kg	M	5.72 - 7.66	6.86±0.75 (7.06)*
	F	6.56 - 12.3	9.20±2.49 (9.88)
API 81-15			
4000 mg/kg	M	6.68 - 9.28	7.94±0.93 (7.94)**
	F	7.28 - 8.54	7.86±0.58 (7.56)*
Cyclophosphamide			
10 mg/kg	M	36.6 - 44.18	40.3±3.53 (38.5)**
	F	18.34 - 31.64	25.5±5.44 (25.06)**

(a) Mean ± standard deviation (Median SCEs/cell)
* $P < 0.05$
** $P < 0.01$ (Mann Whitney test)

It was concluded that the negative and positive controls fulfilled the requirements and that API 81-07 was positive in male mice.

Reliability : (1) valid without restriction

(23)

Type : Dominant lethal assay
Species : Mouse
Sex : Male
Strain : CD-1
Route of admin. : Inhalation
Exposure period : 6 hours each day, 5 days/week for 8 weeks
Doses : Nominal: 0, 100 & 400 ppm. Actual: 0, 98.4 & 378.3 ppm
Result : Negative
Method :
Year : 1980
GLP : No data
Test substance : Jet Fuel A

Method : Groups of twelve male mice (14 weeks old) were exposed by inhalation to nominal vapor concentrations of 100 or 400 ppm of test material. Exposures were for 6 hours a day, five days each week for eight weeks. A control group of mice were placed in the exposure chamber each day and were exposed only to filtered air. On day 40 of the dosing schedule, the animals which served as positive controls received a single i.p. injection of 0.3 mg/kg triethylenemelamine (TEM).
The mice were observed for clinical signs of toxicity twice daily during the exposure phase of the study. The males were then mated with two females for one week. After mating, the females were removed and housed in other cages. After 2 days rest the males were mated with a further two females foe one week. Two weeks after mating, the females were killed using CO_2 and were necropsied. During necropsy, the uteri of each pregnant female were examined and the number of living and dead implants were counted.
The following parameters were calculated:
Fertility index
Computed as No. pregnant females per No. mated females.
Ratio was evaluated by a Chi square test to compare each treatment group and the positive control to the negative control. Armitage's trend for linearity was used to test whether the fertility index was linearly related to arithmetic or log dose.

Total number of implants
This number was evaluated by Student's t-test to determine whether the average number of implantations per pregnant female for each treatment group and positive controls were different to the negative controls.

Dead implants
The dead implant frequencies were transformed (Freeman-Turkey), and the transformed data was compared to the negative control group by Student's t-test

Proportion of females with one or more dead implantations
Proportion of females with two or more dead implantations
These two parameters were evaluated similarly. The quotient was evaluated by the same statistical method used for deriving fertility index with the addition of a probit regression analysis to determine if the probit of the proportions is related to log dose.

Dead implants/Total implants
These were computed for each female and transformed using Freeman-Turkey arc-sine transformation prior to being evaluated by Student's t-test.

Result : There were no deaths during the exposure period, nor were there any Jet-fuel-related clinical signs. Growth rates were unaffected by exposure. The results obtained from the females are as follows.

Negative control		Positive control		100 ppm		400 ppm	
wk1	wk2	wk1	wk2	wk1	wk2	wk1	wk2
Fertility index							
.086	.682	.675	.417	.909	.762	.818	.682
Av. No. implantations/pregnant female							
11.053	12.533	9.048**	8**	12.5	12.375	12	12.6
Average resorptions (dead implants/pregnant female)							
.632	.533	4.619**	5.6**	.95	.875	.222	.4
Proportion of females with one or more dead implantations							
.579	.4	.905**	1.0**	.5	.5	.111	.333
Proportion of females with two or more dead implantations							
.063	.133	.857**	1**	.4*	.125	.056	.067

* P< 0.05
** P< 0.01

With the exception of the proportion of females with two or more dead implantations there were no differences in the developmental parameters in either the one week or two week animals that could be attributable to Jet Fuel A. The one significant finding in the animals mated for one week was considered by the authors to be spurious.
The positive control consistently gave significant increases in the various measured/calculated parameters .
The results support the conclusion that Jet fuel A did not increase the incidence of post-implantation deaths.

Test condition : Jet Fuel A vapor was generated by bubbling dry, oil-free, breathing quality air through a column of liquid Jet fuel A in a 500 ml dust impinger in a heated water bath. The concentrated vapor was diluted with room air in the chamber. The concentration in the chamber was determined at least hourly

The nominal and actual concentrations were:

Concentration (ppm)	
Nominal	Actual
0	0
100	98.4
400	378.3

Reliability : (1) valid without restriction
3 (9)

Type : Dominant lethal assay
Species : Mouse and rat
Sex : Male
Route of admin. : i.p.
Doses : Mouse: 1 ml/kg (diluted 10% in corn oil), rat: Undiluted
Result : Negative

Year : 1973
Test substance : Deodorized kerosene

(2)

Type : Overall summary of in-vivo studies

Remark : Summarized results of all the reported in vivo studies of genotoxicity are shown below

Exposure route	Dosage	Results	Reference
Rat bone marrow cytogenetics			
Hydrodesulfurized kerosene (81-07)			
i.p.	0.3, 1.0 & 3 g/kg	negative	API 32-30240*
Straight run kerosene (83-09)			
i.p.	0.3, 1.0 & 3 g/kg	negative	API 32-31769
Straight run kerosene (8008-20-6)			
i.p. & 0.18 ml/rat	0.02, 0.06 5 consec days	negative	API 26-60017
Straight run kerosene (8008-20-6)			
i.p. & 0.4 ml/rat	0.04, 0.13	negative	API 26-60017
Jet fuel A			
inhalation	100 ppm for 20 days 400 ppm for 5 days	positive	API 27-30051*
Sister chromatid exchange			
Hydrodesulfurized kerosene (81-07			
i.p. mouse	0.4, 2.0 & 4.0 g/kg	positive males only	API 36-30043*
Dominant lethal assay			
Deodorized kerosene			
i.p. Mouse & rat	??	negative	API 26-60098
Jet fuel A			
inhalation Mouse	100 & 400 ppm. 6h/day 8 weeks	negative	API 28-31345*

* Full robust summaries prepared for these studies

5.7 CARCINOGENICITY

Species	:	Mouse
Sex	:	Male
Strain	:	C3H
Route of admin.	:	Dermal
Exposure period	:	104 weeks
Frequency of treatm.	:	2, 4 or 7 days/week
Doses	:	Variable, see methods
Control group	:	Yes, concurrent vehicle
Year	:	1996
GLP	:	Yes
Test substance	:	Straight run kerosene

Method :

The testing of the straight run kerosene (MD-3) was part of an overall larger study. For the purpose of this summary, only the details relating to MD-3 are presented.
The test material was applied to the shorn skin of three groups of 50 male mice for 104 weeks.
The concentration and dosing frequencies were adjusted to ensure that each animal received the same total weekly dose of test material irrespective of dosing frequency. The concentrations and frequencies were selected to determine the influence of skin irritation on the tumorigenic response.

The following dosing regimes were used

Group No.	**Concentration of kerosene* (%)**	**µl/dose**	**Dosing frequency**
9	100	50	2 times/week
10	50	50	4 times/week
11	28.5	50	7 times/week

* Mineral oil was used as diluent
A control group of 50 male mice received 35 µl mineral oil 7 days each week. All animals were observed regularly for viability, clinical signs and a score was given for any dermal irritation that occurred. Body weights were recorded throughout the study.
When they developed, dermal growths were measured and documented.
All animals were necropsied either when they died during the study or at the end of the study. The necropsy included an examination of the body, all orifices and the carcass, cranial, thoracic and abdominal cavities, including their contents. For all animals, tissues were preserved and examined microscopically from all skin tumors, skin from treated and untreated sites and any grossly observable masses.

Remark :

The study was designed to assess the carcinogenic potential of middle distillates under conditions in which irritation was present or absent.

The results demonstrated that undiluted kerosene applied twice weekly to the skin caused irritation and skin tumors developed in 12 of 50 mice.
In the groups where no irritation occurred, no tumors developed either even though the same total weekly dose of test material was applied to the skin as had been the case with the undiluted material.

Result :

Survival was less in the 100 % MD-3 treated group compared to the negative controls.
Dermal irritation occurred in the groups exposed to kerosene.
The dermal irritation scores were:

Group	Range of scores	Mean dermal score
Negative control (oil)	0-0.22	0.06

100% kerosene 2X/week	0-4.0	2.92
50% kerosene 4X/week	0-0.8	0.09
28.5% kerosene 7X/week	0-0.2	0.04
Positive control (HCO)	0-2.0	0.73

There were no other treatment-related clinical findings.
Treatment did not have any adverse effect on body weights.
Treatment-related findings at post mortem were limited to dermal irritation and were consistent with the findings of the in-life phase of the study.
Liver masses and gastrointestinal abnormalities were observed but these were found in all groups and were considered to be incidental.
Tumors developed in the positive control group (HCO) and only in the MD-3 group that received undiluted sample twice weekly as follows:

Material	**Oil**	**HCO**	**MD-3**	**MD-3**	**MD-3**
Concentration			28.5%	50%	100%
No. applications/week			7	4	2
No. mice examined	50	50	50	50	50
No. mice with tumors	0	47	0	0	12
Tumor types					
Squamous cell carcinoma	0	42/73*			7
Spindle cell carcinoma	0	0	0	0	1/2*
Fibrosarcoma	0	0	0	0	3/5*
Melanoma	0	0	0	0	0
Papilloma	0	37/88*	0	0	6

* / = No with neoplasms/actual incidence of neoplasms

Test substance : The sample of straight run kerosene was designated sample MD-3 and had been characterized as follows (CONCAWE 91/51):

CAS No.	64742-81-0
Crude source	Middle East
Kinematic viscosity (cSt)	1.26
Flash point (°C)	49 fire
Distillation range (°C)	175-237 (10 and 90%)
Sulfur content (%)	0.14
Nitrogen content (mg/l)	1.3
Relative density	0.7978
Aniline point	61.4
Aromatics (%)	17.1
Olefins (%)	1.7
Saturates (%)	81.2
PAC 3-7 rings (% wt)	0

Reliability : (1) valid without restriction

(37) (47)

Species : Mouse
Route of admin. : Dermal

Remark : The results of several carcinogenicity studies have been reported for kerosene streams and jet fuels.
These data have been reviewed and summarized elsewhere (CONCAWE, 1995; IARC, 1989; ASTDR, 1998).
The study described in the summary above is considered to be the most recent and the most robust study. However, for completeness, other studies that have been reported are summarized in the following table (taken from CONCAWE, 1995).

Dosing regime	Duration	Result* Tumors	Latency (weeks)	Reference
Straight-run kerosene (API 83-09)				
50 µl, 2x/week				
50 mice	>2 years	19 malignant 1 benign	76	API 36-33220
Hydrodesulfurized kerosene (API 81-07)				
50 µl, 2x/week				
49 mice	> 2 years	26 malignant 1 benign	77	API 36-31364
Straight run middle distillate (from naphthenic crude)				
50 mg, 2x/week				
50 mice	102 weeks	14/30	70	API 32-30964
Straight run middle distillate from paraffinic crude				
50 mg, 2x/week				
50 mice	82 weeks	13/27	62	API 32-30964
Jet fuel A				
25 mg, 3x/week				
50 mice	105 weeks	11/43	79	Clark 1988
Jet fuel JP-5				
500mg/kg				
5x/week	103 weeks	No tumors		NTP 1986
100 mice				

It should be noted that in those studies in which tumors developed, moderate to severe skin irritation was also observed. Since the materials contain very low or no PACs, it was suggested that tumor development may have resulted from chronic skin irritation. CONCAWE, therefore, carried out a program of studies that examined the effect of skin irritation on the tumorigenicity of kerosene (CONCAWE 96/62). In the CONCAWE study, it was found that in the absence of skin irritation kerosene skin tumors did not develop.
Jet Fuel A was included in another study, designed evaluate the role of skin irritation in a skin carcinogenicity of middle distillates. In this study, Jet fuel A was applied either twice weekly or intermittently in which dosing was suspended when marked signs of dermal irritation were noted. When the Jet Fuel A was applied three times weekly 44% of the mice developed tumors and this incidence was reduced to 2% in those animals in which dosing was intermittent. The authors concluded that chronic skin irritation may be a necessary, but not sufficient, condition for skin tumorigenicity (Freeman et al, 1993).

In a review of the available information on the carcinogenicity of middle distillate fuels, Nessel (1999) concluded that the tumorigenic activity of the fuels was secondary to skin irritation.

(19) (25) (27) (35) (40) (53) (60)

5.8.1 TOXICITY TO FERTILITY

Type	:	Reproduction/developmental study
Species	:	Rat
Sex	:	Female
Strain	:	Sprague-Dawley
Route of admin.	:	Dermal
Exposure period	:	14 days premating to day 20 of gestation
Frequency of treatm.	:	Daily, 7 days/week
Premating exposure period		
Male	:	14 days
Female	:	14 days
Doses	:	165, 330 & 494 mg/kg/day
Control group	:	Yes
other: NOAEL reproductive/developmental toxicity	:	494 mg/kg bw
Method	:	OECD Guide-line 421
Year	:	1997
GLP	:	No data
Test substance	:	Hydrodesulfurized kerosene

Method :

The study was performed in accordance with OECD guideline 421 with the addition that males were treated for 8 weeks to improve observation of effects on the reproductive system. Also females were weighed 7 times during gestation rather than 4, and at necropsy, 7 organs in addition to the reproductive organs were weighed.

Ten male Sprague Dawley rats (aged approximately eight week old, weighing 275-285g) and 10 females (same age and weighing 183-187g) were treated dermally with kerosene at concentrations of 0 (sham-treated and vehicle control groups), 20, 40 or 60% (v/v) in mineral oil in a dosing volume of 1 ml/kg. Dosage equivalents were 0, 165, 330 and 494 mg/kg. These doses were selected on the basis of the results of a preliminary 2-week range finding study.
Test material was applied daily to the shorn skin of the animals 7 days/week from 14 days premating, during 14 days mating and through 20 days of gestation. Collars were fitted to the animals during the dosing period to prevent ingestion of applied materials. After the final dose, the collars were removed and residual test material was wiped from the skin. Males continued treatment through gestation until final female sacrifice on days 4-6 of lactation.
There were two control groups: the vehicle control was given mineral oil only and in the sham-treated group the animals had been fitted with collars and were stroked with the tip of a syringe, but no material was applied.

During the mating period the test material remained on the animal's backs for 6 hours. Prior to pairing, the test material was removed by wiping. Rats were mated overnight on a 1:1 ratio and were separated the following morning. Collars were then applied prior to the next dose being applied. Females were monitored for evidence that mating had taken place.

Pregnancy was determined by the presence of a vaginal plug or sperm in a vaginal lavage sample. If observed, the female was considered to be at day 0 of gestation. Any female that did not show evidence of mating was placed with the same male the following evening. Any female that did not show evidence of mating at the end of a 2 week mating period was presumed pregnant (gestation day 0 = last day of cohabitation).

Animals were checked twice daily for morbidity and mortality during weekdays but only once daily at weekends. Animals were also observed immediately prior to dosing and after the last animal had been dosed for appearance, behavior and motor activity, respiratory function, central nervous system function, excretory function and biological discharges. Effects of test material on the skin were assessed and scored weekly, using Draize scales for erythema and edema and for chronic deterioration. Males were weighed on the first day of dosing, then weekly and on the day of sacrifice. Females were also weighed on the first day of dosing, then weekly until mating was confirmed and thereafter on gestation days 0, 3, 6, 10, 13, 16 and 20 and on post partum days 0 and 4. Food consumption was also monitored on a similar schedule except through the mating period.

Each presumed-pregnant female was observed daily from gestation day 20 for parturition; evidence of dystocia was noted. The day of delivery was designated postpartum day 0. Maternal behavior and appearance were monitored daily until sacrifice.
Each litter was examined as soon as possible after birth to establish the number and sex of pups, stillbirths, live births and the presence of gross abnormalities. Pups were examined daily for presence of milk in their stomachs. Any pup found dead was examined externally and unusual findings were recorded. The body weight of each viable offspring was individually measured and recorded on post partum days 1 and 4.

Adult females that did not deliver were sacrificed on day 25 of gestation. Dams that delivered and maintained their litters until post partum day 4 were sacrificed with their offspring on post partum days 4-6.
All males were sacrificed after the females had been killed.
All animals were examined macroscopically for structural anomalies and pathological changes, with emphasis on the reproductive organs. The numbers of implantation sites and corpora lutea of each adult female was recorded. No tissues from offspring were retained.

The liver, kidneys, adrenals, thymus, spleen, brain and heart of all parental animals were weighed. In addition the testes and epidiymides of parental males were weighed.
Skin from treated sites, ovaries and testes and epididymides were prepared for histological examination. Pathological evaluation was performed on reproductive organs from all males and pregnant females in both control groups and the high dose group and on treated skin from all groups.

Statistical evaluation
Quantitative data (body weight and food consumption) were analyzed by parametric methods: analysis of variance (ANOVA) and associated F-test, followed by Dunnet's test for multiple comparisons, provided there was statistical significance in the ANOVA. Maternal reproductive data were evaluated by ANOVA followed by group comparisons using Fisher's exact test. Differences between control and treatment groups were considered

statistically significant only if the probability of the differences being due to chance was less than 5% (P< 0.05).

Remark : Two preliminary studies were carried out before the reproductive/developmental toxicity study was conducted.

A dermal irritation study to determine an appropriate dosing regime for the main study

A study of percutaneous absorption to ensure that dilution of test material with mineral oil did not prevent percutaneous absorption of kerosene components.

Neither of these studies are summarized here. A summary is only provided for the reproductive/developmental toxicity study itself.

Result : One pregnant mid-dose female died before delivery. No other animals died or were prematurely sacrificed and no clinical signs of toxicity were observed.
Skin irritation among males varied from slight to moderate with increasing dose and was most severe in the high dose group. Mild to moderate skin irritation was observed in females at the highest concentration.
At terminal sacrifice, no findings were reported except for those on the skin. Microscopic changes were found in the skin of males in the vehicle control and all kerosene-treated groups. In females changes were only observed in the high dose group animals. The skin findings (macroscopic and microscopic) are shown in the following table.

			Kerosene (mg/kg)		
Parameter	**Control**	**Mineral oil**	**165**	**330**	**494**
Males					
No animals	10	10	10	10	10
Max. skin irritation score, sum of means					
Week of max severity					
	-	2	2	5	5
Mean (SD)	0	1.3(1.2)	2.4(0.7)	2.5(2.0)	3.3(2.1)
Min/max score	0	0/3	1/3	0/7	1/7
Gross necropsy observations					
Crust/scab	1	0	0	0	1
Scaly/dry/flaky	0	0	1	2	3
Histopathological observations					
Acanthosis/hyperkeratosis					
	2	5	8	7	8
Hyperplasia, sebaceous glands					
	3	5	5	3	5
Inflammation, dermal					
	2	1	6	6	7
Necrosis, epidermal, focal					
	1	0	1	1	5
Females					
No animals	10	6	10	10	10
Max. skin irritation score, sum of means					
Week of max severity					
	6	7	3	4	4
Mean (SD)	0.2(0.6)	0.7(1.0)	0.4(0.8)	1.1(0.9)	2.3(1.8)
Min/max score	0/2	0/2	0/2	0/2	1/7
Gross necropsy observations					
Crust/scab	0	1	1	0	3
Scaly/dry/flaky	0	0	0	0	0
Histopathological observations					
Acanthosis/hyperkeratosis					
	3	2	5	5	6

Hyperplasia, sebaceous glands					
	1	0	0	0	1
Inflammation, dermal					
	0	1	1	1	4
Necrosis, epidermal, focal					
	0	0	0	0	0

Body weights were unaffected by treatment. However over the course of the 8 weeks, high dose males gained less weight than the controls (201 g compared to 237g for the controls). Food consumption was unaffected by treatment.
High dose males had a higher mean relative kidney weight than controls (0.76 vs 0.66). This was attributed to the lower mean final body weights of the high dose group. No other organ or organ/body weight changes were recorded.

	Controls		**Kerosene (mg/kg)**		
Parameter	**Sham**	**Oil**	**165**	**330**	**494**
No animals	10	10	10	10	10
Fertility index	100%	90%	90%	80%	100%
Litter with liveborn pups					
	10	9	9	7[a]	10
Corpora lutea					
Number	169	151	158	122	172
Mean	16.9	16.8	17.6	17.4	17.2
(SD)	(1.9)	(2.4)	(2.0)	(0.8)	(2.9)
Implantation sites					
Number	163	149	155	18	167
Mean	16.3	16.6	17.2	16.9	16.7
(SD)	(1.9)	(2.4)	(1.8)	(1.3)	(2.8)
Pups delivered					
Total	152	131	147	109	150
Mean	15.2	14.6	16.3	15.6	15.0
(SD)	(2.0)	(2.7)	(2.3)	(2.9)	(2.9)
Liveborn	152	130	143	108	148
Livebirth index	100%	99%	97%	99%	99%
Pups dying					
day 0	3	0	1	1	1
days 1-4	2	4	1	1	9[bc]
Pups surviving					
4 days	147	126	141	106	138
Viability index	97	97	99	98	93[c]
Pup weight/litter (g)					
day 1 mean	6.9	6.8	7.0	7.0	6.7
day 4 mean	9.9	9.6	10.1	9.9	9.8

[a] one dam died on gestation day 21; the cause of death was unrelated to treatment

[b] significantly different from control (P<0.05)

[c] One dam had a malfunctioning water bottle; when 4 dead pups from this litter are excluded from the analysis, no significant difference

from control was found.

No test-material-related microscopic changes were observed in the testes or epididymides of adult male rats or in the ovaries of adult female rats.

Test substance : The chemical composition of the sample of hyrdodesulfurized kerosene was determined by ASTM method D 1319-1 and the results are tabulated below.

Component	Weight %
Nonaromatics	80.27
Saturates	78.61
Olefins	1.66
Aromatics	19.72
<3-ring PAC	>19.72
3-7 -ring PAC	<0.01

Reliability : (1) valid without restriction

(64)

5.8.2 DEVELOPMENTAL TOXICITY/TERATOGENICITY

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : Six hours each day
Frequency of treatm. : Daily
Duration of test : Gestation days 6 through 15
Doses : Nominal: 100 & 400 ppm, Actual: 106 & 364 ppm
Control group : Yes
NOAEL maternal tox. : 364 ppm
NOAEL teratogen. : 364 ppm
Year : 1979
GLP : Yes
Test substance : As prescribed by 1.1 - 1.4

Method : Groups of 20 female presumed-pregnant rats aged 12 weeks were exposed to nominal concentrations of either 100 or 400 ppm kerosene vapor. Exposures were accomplished in chambers for 6 hours each day on days 6 through 15 of gestation. A control group of 20 presumed-pregnant rats of the same age served as controls and were placed in chambers and exposed to room air only.
The rats were weighed on days 0, 6, 15 and 20 of gestation and food consumption was measured throughout the study.
The animals were also observed daily for clinical signs.

On day 20 of gestation, the animals were anesthetized with chloroform and the visceral and thoracic organs were examined. The uterus was removed and opened and the number of implantation sites and their placement in the uterine horns were recorded. Live and dead fetuses and resorption sites were also recorded and the fetuses were removed, examined externally for abnormalities and weighed.

One third of the fetuses were fixed in Bouin's fluid and were examined subsequently for changes in the soft tissues of the head, thoracic and visceral organs. The remaining fetuses were stained with Alizarin red S and examined subsequently for skeletal abnormalities.

Statistical analysis
Analysis of the data was performed using the litter as a basic sampling unit. Dunnett's t-test was used to determine statistical significance (P<0.05) with regard to differences between means with near-normal distribution (body weights and food consumption of dams, mean pup weight based on litter averages). Ratios (nidation index and implantation/corpora lutea ratio) were analyzed with a 2 x 2 contingency table with Yates' correction. Wilcoxon Rank Sum was used for discontinuous parameters as measured by the number of abnormal fetuses within a litter.

Result **:** There were no compound-related deaths or clinical signs throughout the study.
At necropsy two animals in the 100 ppm group had lung mottling, but this was considered to be an incidental finding.
There were no significant differences in either body weight or food consumption data.
The following is a summary of reproductive data base on observations of the uterine contents on day 20 of gestation

		Dose (ppm)		
Observation	**Historical control***	**0**	**100**	**400**
Nidation index (females with implantations/Bred				
	55/61	19/20	18/20	18/20
Females dying prior to Cesarean section				
	0	0	0	0
Live litters	54	19	18	18
Implantation sites (left horn/right horn)				
	301/363	110/118	106/138	126/114
Resorptions				
Total	43	18	21	12
Litters	24	11	10	7
Dead fetuses				
Total	0	0	0	0
Litters	0	0	0	0
Mean live litter size (fetuses)				
	11.3	11.1	12.4	12.7
Average fetal weight (g)				
	4.1	4.3	4.1	3.9

* Based on 54 litters

Examination of offspring at delivery did not reveal any treatment-related abnormalities.
Examination of Bouin's fixed specimens did not reveal any treatment-related abnormalities.
The sex ratio was also unaffected by treatment.

Skeletal examinations revealed the following:

Dose group	**No. fetuses examined**	**No. fetuses normal**	**Fetuses common* changes**	**with unusual skeletal variations**
0	140 (19)	60	72 (16)	8 (4)
106.4	150 (18)	69	72 (15)	9 (4)
364	154 (18)	62	84 (18)	8 (5)

* Fetuses with commonly-encountered changes only
The authors comment that most of the changes, while not strictly normal, are frequently observed in 20-day-old rat fetuses of this strain and source

in their laboratory.
The changes were not malformations but were mostly related to retarded bone ossification. Neither the frequency nor the character of the changes indicated an adverse effect on fetal growth and development or a teratogenic potential.

Test condition : A vapor of the test material was generated by metering it into a warmed flask and passing compressed air through the flask. This concentrated vapor was further diluted with room air as it entered the chamber.
The chamber concentrations were monitored hourly throughout the exposure.

Nominal and mean actual chamber concentrations are as shown:

Chamber concentration (ppm)	
Nominal	**Actual**
0	0
100	106.4 ± 10.23
400	364 ± 37.53

Reliability : (1) valid without restriction

(7)

Species : Rat
Sex : Female
Strain : Sprague-Dawley
Route of admin. : Inhalation
Exposure period : Six hours each day
Frequency of treatm. : Daily
Duration of test : Days 6 through 15 of gestation
Doses : Nominal: 100 & 400 ppm, Actual: 102.5 & 394.7 ppm
Control group : Yes
NOAEL maternal tox. : 394.7 ppm
NOAEL teratogen. : 394.7 ppm
Year : 1979
GLP : Yes
Test substance : Jet fuel A

Method : Groups of 20 female presumed-pregnant rats aged 12 weeks were exposed to Jet Fuel A at nominal concentrations of either 100 or 400 ppm vapor. Exposures were accomplished in chambers for 6 hours each day on days 6 through 15 of gestation. A control group of 20 presumed-pregnant rats of the same age served as controls and were placed in chambers and exposed to room air only.
The rats were weighed on days 0, 6, 15 and 20 of gestation and food consumption was measured throughout the study.
The animals were also observed daily for clinical signs.

On day 20 of gestation, the animals were anesthetized with chloroform and the visceral and thoracic organs were examined. The uterus was removed and opened and the number of implantation sites and their placement in the uterine horns were recorded. Live and dead fetuses and resorption sites were also recorded and the fetuses were removed, examined externally for abnormalities and weighed.

One third of the fetuses were fixed in Bouin's fluid and were examined subsequently for changes in the soft tissues of the head, thoracic and visceral organs. The remaining fetuses were stained with Alizarin red S and examined subsequently for skeletal abnormalities.

Statistical analysis
Analysis of the data was performed using the litter as a basic sampling unit. Dunnett's t-test was used to determine statistical significance (P<0.05) with regard to differences between means with near-normal distribution (body weights and food consumption of dams, mean pup weight based on litter averages). Ratios (nidation index and implantation/corpora lutea ratio) were analyzed with a 2 x 2 contingency table with Yates' correction. Wilcoxon Rank Sum was used for discontinuous parameters as measured by the number of abnormal fetuses within a litter.

Result **:** There were no test material-related deaths during the study.
Eye irritation (or infection) occurred in a dose-related manner. The irritation consisted of discharge from the eye, swollen eyelids or swollen areas around the eye. The signs of irritation lasted from 2 to 8 days with most animals showing signs for 3 days.
Irritation occurred at the following frequency.
Control group 2/20 animals
100 ppm group 7/20 animals
400 ppm group 20/20 animals

Body weights and food consumption were unaffected by treatment.

At necropsy the following findings were made.
Controls 1/20 with mottled lungs
1/20 with distended intestines
1/20 with fluid-filled and distended uterus

100 ppm group 2/20 with pale lungs, mottling or dark foci
One of these animals had a fluid-filled uterus
1/20 fluid-filled cyst on and around right ovary
1/20 enlarged, fluid-filled cavernous kidneys

400 ppm group 1/20 granular spleen
1/20 granular liver
1/20 mottled lungs and fluid-filled uterus

Observations of the uterine contents did not reveal any treatment-related effects. The data are tabulated below.

		Dose (ppm)		
Observation	**Historical control***	**0**	**100**	**400**
Nidation index (females with implantations/Bred				
	--	12/20	12/20	16/20
Females dying prior to Cesarean section				
	--	2**	0	0
Live litters	99%	12	12	16
Implantation sites (left horn/right horn)				
	46%/54%	64/81	67/88	97/108
Resorptions				
Total	326	8	8	9
Litters	51%	6	4	7
Dead fetuses				
Total	1	0	0	0
Litters	1	0	0	0
Live fetuses/Implantation site				
	92%	137/145	147/155	197/205***
Mean live litter size (fetuses)				
	12.2	11.4	12.3	12.3
Average fetal weight (g)				
	3.6	4.0	3.6	3.7

* Based on 328 litters

** Misdosed and killed
*** Includes set of twins on a single placenta

Examination of offspring at delivery did not reveal any treatment-related abnormalities.
Examination of Bouin's fixed specimens revealed three fetuses from two litters in the 100 ppm group which were judged to be smaller than normal. Otherwise, there were no other treatment-related abnormalities.
The sex ratio was also unaffected by treatment.

Skeletal examination revealed the following:

Dose group	No fetuses examined	No. fetuses normal	Fetuses common* changes	with unusual skeletal variations
0	90 (12)	44	41 (11)	5 (4)
102.5	98 (12)	62	32 (10)	4 (3)
394.7	131(16)	51	64 (15)	16 (6)

* Fetuses with commonly-encountered changes only

The authors comment that most of the changes, while not strictly normal, are frequently observed in 20-day-old rat fetuses of this strain and source in their laboratory.
The changes were not malformations but were mostly related to retarded bone ossification. Neither the frequency nor the character of the changes indicated an adverse effect on fetal growth and development or a teratogenic potential.

Test condition : The sample of Jet Fuel A was analyzed and the following results were obtained.

Parameter	Method	Result
Flash point	ASTM D 56	135°F
Boiling range	ASTM D 86	325-540 °F
Sulfur		0.036 wt %
Benzene		0.006 vol. %
Hydrocarbon types by mass spectrometry ASTM D2425 (vol. %)		
Saturates		
Paraffins		40.6
Monocycloparaffins		27.3
Dicycloparaffins		10.9
Tricycloparaffins		3.3
Total saturates		82.1
Aromatics		
Alkylbenzenes		10.0
Indans & Tetralins		3.6
Dinaphtheneobenzenes		0.9
C10 Naphthalenes		0.2
C11 Naphthalenes		0.1
Biphenyls, etc.		0.1
Fluorenes, etc.		0
Tricyclic aromatics		0
Total aromatics		17.9

A vapor of the test material was generated by metering it into a warmed flask and passing compressed air through the flask. This concentrated vapor was further diluted with room air as it entered the chamber.
The chamber concentrations were monitored hourly throughout the

exposure.

Nominal and mean actual chamber concentrations are as shown:

Chamber concentration (ppm)	
Nominal	**Actual**
0	0
100	102.5 ± 5.7
400	394.7 ± 19.3

Reliability : (1) valid without restriction

(6)

5.9 SPECIFIC INVESTIGATIONS

Endpoint : Tumorigenicity
Type : Initiation/Promotion assay
Species : Mouse
Sex : Male
Strain : CD-1
Route of admin. : Dermal
Vehicle : Acetone
Control group : Yes
Year : 1989
Test substance : API 81-07

Method : The initiating and promoting activity was determined for nine different petroleum streams, including kerosene (sample API 81-07), in this study. Male CD-1 mice approximately 6 weeks old were used.

Test for initiating activity
50 µl API 81-07 was applied on five consecutive days to the shorn skin of 30 male mice. After a two week rest period, 50 µl of the promoter PMA (0.1 mg/ml) was applied topically to each animal, twice weekly for 25 weeks. Following the last application of PMA, the dosing was terminated and the mice were sacrificed. A limited gross necropsy was carried out on all mice.
A group of 30 mice treated only with acetone served as the negative controls.
A positive control group were given DMBA. Mice in this group received only one initiating dose (50 µl of 1mg/ml in acetone) on day five of the study. Thereafter, promotion with PMA was the same as for the test groups.

Test for promoting activity
A single 50 µl dose of DMBA (1mg/ml in acetone) as initiating agent was applied to the shorn skin of a group of 30 male mice. After a two week rest period API 81-07 was applied twice weekly to the mice for 25 weeks.
The negative control group received only acetone as a single dose as the initiating dose. This was followed with 25 weeks of treatment with acetone twice weekly.
A further control group received a single dose of DMBA and following a two week rest these mice were sham handled. This group was included to demonstrate that DMBA had been applied at a sub-threshold dose.
After the completion of the promotion phase all animals were sacrificed and were subjected to a limited gross necropsy.

Throughout the study, the mice were observed daily for morbidity and

mortality. Animals were also observed for mass formation throughout the study.
All mice, whether dying or killed in extremis, were subjected to a limited gross necropsy. This consisted of: examination of application site, brain, heart, lungs, liver, kidneys, spleen, testes, urinary bladder, stomach, duodenum, jejunum, ileum, cecum and colon. Skin of the application site was removed, fixed and processed for histological examination. Other lesions of the internal organs were recorded but no further investigation was undertaken.

Result : The results demonstrated that API 81-07 was not a tumor initiating agent but did show tumor promoting activity.

The results of the study are summarized in the following table.

No. mice	Masses observed In life	Masses observed Histopath	Latency (weeks) First	Latency (weeks) Mean mass	Latency (weeks) Median
INITIATION ASSAY					
API 81-07					
30	3	3	25	26.7	27
DMBA control					
30	30	30	9	10.6	10
Acetone control					
30	3	3	16	18.5	18.5
PROMOTION ASSAY					
API 81-07					
acetone					
30	0	0	0	0	0
DMBA					
30	21	22	14	20.2	19
Sham control					
DMBA					
29	0	0	0	0	0

Remark : In another study, the role of chronic acanthosis on tumor promotion by hydrodesulfurized kerosene (API 81-07) was studied. The authors concluded that their results suported the hypothesis that induction of a lasting, albeit mild, hyperplasia is an essential but not sufficient requirement for tumor promotion. They also concluded that subacute inflammation did not appear to be a significant factor in tumor promotion by the kerosene tested (Skisak, 1991).

(26) (72)

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American Petroleum Institute Report No. 26-60098

(3) API (1977)
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American Petroleum Institute Med. Res. Publ. 26-60017

(4) API (1978)
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American Petroleum Institute HESD Publ. 26-60103

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(15) API (1985)
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American Petrleum Institute report 33-30443

(16) API (1985)
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acute dermal toxicity study in rabbits
primary dermal irrritation study in rabbits
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